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TABLE OF CONTENTS

A vascular disease of Gladiolus caused by Fusarium. LUCIA MCCULLOCH 263

Quantitative studies with carborundum and its use in local-lesion tests ..... A. S. COSTA 288

Studies of stem rust (Puccinia graminis) from Poa ampla, Avena fatua, and Agropyron spicatum in the Pullman, Washington, region ..... GEORGE W. FISCHER AND CARL E. CLAASSEN 301

Chemical and physical characteristics of maize cobs in relation to the growth of Nigrospora oryzae ..... J. H. STANDEN 315

Two new viruses affecting tobacco and other plants. KENNETH M. SMITH AND ROY MARKHAM 324

Pollen as a source of walnut bacterial blight infection ..... P. A. ARK 330

A virus disease of lovage (Ligusticum scoticum). KENNETH M. SMITH AND ROY MARKHAM 335

"Freckle," a spotting of tomato fruits ..... H. REX THOMAS 341

Mature peach fruits affected by leaf curl ..... E. M. HILDEBRAND 345

Relation of rust damage in seed flax to seed size, oil content, and iodine value of oil ..... H. H. FLOR 348

Bacterial soft rot of Sansevieria ..... J. G. BROWN AND ALICE M. BOYLE 350

Phytopathological Notes 352

The pathogenicity of a nonsporulating basidiomycete on grasses in Minnesota ..... EDWARD A. ANDREWS

The production of healthy shoots by wilted tomato plants. DAVID GOTTLIEB

Low-lime Bordeaux mixture controls leaf gall on Azaleas. JOHN R. COLE

The nature of resistance of flax to Fusarium lini. MAX SCHUSTER

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A VASCULAR DISEASE OF GLADIOLUS CAUSED BY FUSARIUM

LUCIA MCCULLOCH[1]

(Accepted for publication July 17, 1943)

INTRODUCTION

A Fusarium disease of gladiolus commonly known as yellows, wilt, or core rot, scarcely known and certainly not serious in 1920, has now spread to most if not all producing regions in the United States and is generally considered the most serious disease of gladiolus. The Fusarium wilt disease, deep seated in the interior of the corms, easily escapes notice. Since symptoms become visible at the surface of the corm only in advanced stages of the disease, infected corms are often shipped and planted without any suspicion of their condition.

The present publication is based upon 18 years of investigation of various phases of the disease.

HISTORY OF THE DISEASE

In 1923 a large number of gladiolus corms of the Nanus type (Bride, Blushing Bride, Peach Blossom, Ruby, Rosy Gem, San Mateo, and others) were received from two localities in California. Although the corms were clean, bright, and normal in exterior appearance, they showed on cutting 90 per cent interior rot (Fig. 1, *A, B*). The rot varied from a slight discoloration in the basal scar to browning of the entire core and radiating fibro-vascular strands. From these corms a *Fusarium* was isolated. From later shipments from the same localities the same *Fusarium* was consistently isolated, and inoculation experiments proved that this organism was capable of producing an internal rot of Nanus varieties and similar symptoms in the Primulinus and Grandiflorus types.

In the following year, Nanus, Primulinus, and Grandiflorus types of gladiolus affected with the same vascular necrosis were received from several western areas and in 1925 and 1926 similar specimens came from States as widely separated as North Dakota, Mississippi, and New Jersey. The progress of the disease seemed definitely from the West with prevalence increasing each season. Two of the California growers stated in 1923 that for several years they had imported stock from Holland. In 1926 the writer found the disease in the variety Odin in a shipment from Holland. Again in 1927 in another shipment from Holland the variety Odin and a Nanus type, Bride, had typical symptoms of the disease. From all of these specimens the usual *Fusarium* was isolated.

In the literature there are numerous references to Fusarium diseases of gladiolus but with descriptions not always sufficient for identification. The earliest reference found by the writer that seems to describe the disease

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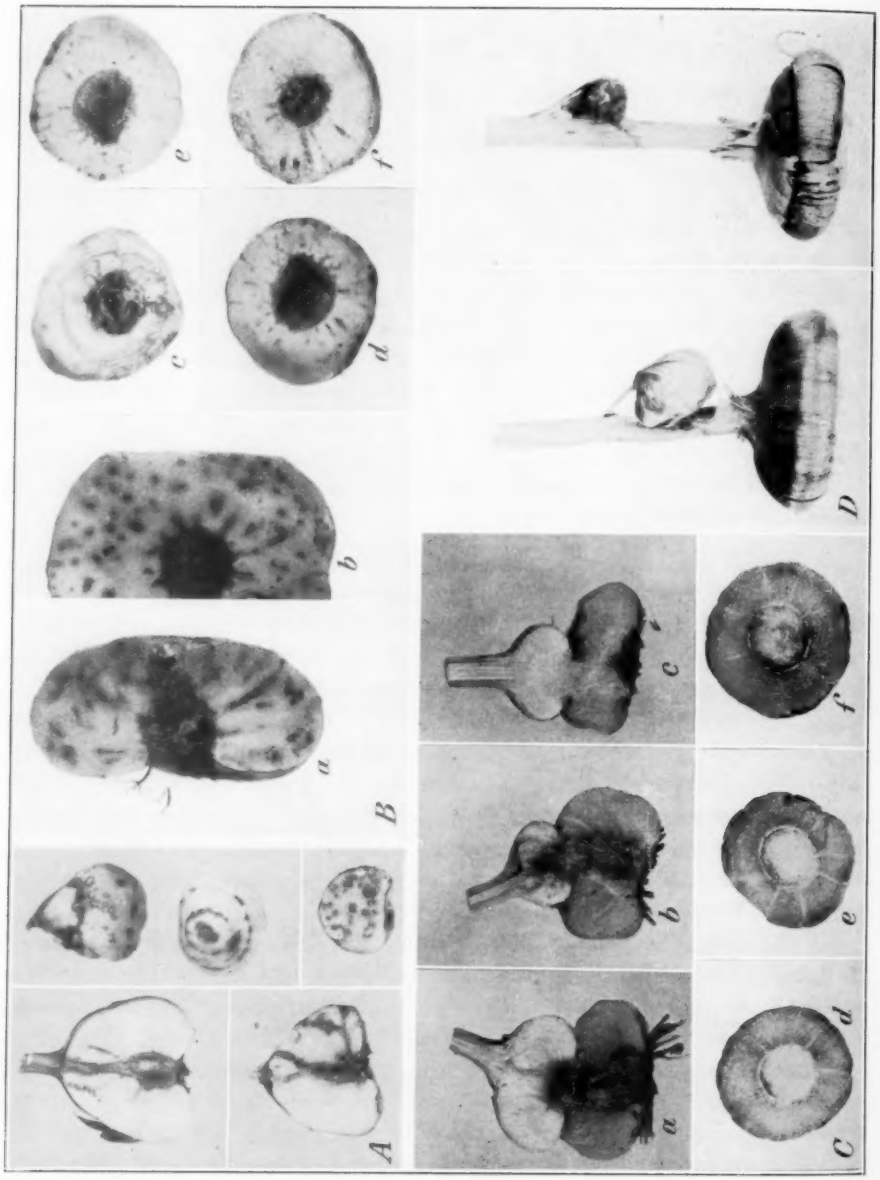

FIG. 1. Vascular infection of gladiolus corms. *A*. Nanus varieties: longitudinal and transverse sections and exterior of *Fusarium*-infected corms from California in 1923. *B*. *a*, Longitudinal; *b*, transverse sections of a corm from storage in midwinter; *c*, base of an infected corm; *d–f*, transverse sections of *c* at different levels. *C*. *a* and *b*, Extension of infection from the parent corm into the new corm; *c*, healthy corm; *d*, slightly infected cylinder; *e*, nearly half of vascular cylinder infected; *f*, entire cylinder infected. *D*. Aerial corms collected in October on current growth. All × ½ natural size.

clearly is a note by a California grower, W. A. Pryal (18), who described an interior corm rot and leaf yellowing of gladiolus in 1909. Massey, in 1922 (7), published a brief note, and, in 1926 (8), a full description of a Fusarium decay of gladiolus corms. Van Poeteren (25) reported the vascular disease as present in Holland as early as 1925. A dry rot of gladiolus caused by *Fusarium* was reported from England in 1927 (2), and a vascular Fusarium disease in 1939 (13). The vascular type was reported from California in 1927 (20). Later accounts of the vascular disease are found in reports by Bellard (3), Dimock (4), Nelson (14, 15, 16, 17), the writer (9, 11), and others (1).

SYMPTOMS

Leaf Symptoms

In infected Nanus gladiolus, apparently normal plants suddenly wilt or turn yellow and die prematurely. In the larger types of gladiolus the stout leaves do not wilt easily and wilting is less noticeable, often absent. The falling over of the whole plant is due more to destruction of tissues at or near the ground level than to wilting from lack of water.

When infected stock is planted, the more seriously diseased corms rot, without sprouting, or produce feeble shoots that soon die. Slightly infected corms often grow fairly well, some even producing a new corm. When healthy stock is planted in infested soil, all or most of the corms germinate and the plants for a time appear normal but soon show stunting and loss of color. With either infected stock or infested soil the end results of stunting, yellowing, and drying of the foliage are similar. The roots of such plants show various degrees of discoloration and rot. In advanced stages of disease the roots are absent or reduced to the central vascular strand. In the corms the infection may be slight and only in the basal scar or the entire core and vascular strands (Fig. 1, *B, C*) may be decayed.

If the plants are fairly well grown before the infection becomes serious, the leaf yellowing is correspondingly delayed or even absent. In young, tender leaves, wilt may occur before the leaves yellow. With a uniform infection of the whole vascular system, all of the leaves become yellow and dry, the change usually beginning at the tip and proceeding regularly downward. Sometimes leaf yellowing shows first in the lower leaves, and in some partially resistant varieties a slight wilting and yellowing of the first leaf is the only indication of infection. Corms infected in only a section of the root plate show correspondingly less yellow in the leaves. Probably the yellowed leaves are in vascular connection with infected roots, but it is difficult to establish this.

In very susceptible varieties the foliage symptoms appear very early. In heavily infested soil and with adverse cultural conditions, even the resistant varieties sometimes become slightly infected, but such infection produces very slight evidence of disease in the corms and usually no noticeable foliage symptom. Intermediate between the very susceptible and the resistant

varieties are those in which more or less infection occurs without disease symptoms in the leaves.

The leaves of some varieties (Hopi, Los Angeles, Maid of Orleans, Alice Tiplady, and others) turn yellow naturally with maturity, with appearance very similar to yellows, but examination of the roots and corms shows no trace of disease. Furthermore, diseases other than Fusarium yellows may cause yellowing, so that this is not an entirely dependable symptom.

Corm symptoms

In corms the disease symptom varies from a slight discoloration at the base to complete rot. Extensive interior rot may exist without any outward sign of disease. When infected plants are harvested, the new corms may appear entirely normal, but, when cut, usually show a surprising amount of brown rot in the interior (Fig. 2, *A*). In typical average stages of disease both the parent and the new corms have discolored cores and radiating vascular strands. In a more advanced stage the infected strands reach the surface of the corm at the nodes where brown lesions develop (Fig. 2, *E, a–b*). If this stage of infection develops before the corm is dug and the husks are not too dry, the infection spreads into the vessels of the husk (Fig. 2, *E, c*). The node lesions are more often found in corms in storage. These surface lesions are bright dark-brown, with margins of light reddish-brown. However, there is more or less variation in the different varieties of gladiolus.

Infection may exist in a slightly broken basal scar, perhaps with no discoloration. Sometimes the parent corm shows less disease than the new corm. In severe infections, especially if the soil is moist, the parent corm is completely rotted by harvest time instead of being normally dry and shriveled. In such cases the new corm is usually in an advanced stage of rot (Fig. 2, *A*). Sometimes new corms are infected directly from contractile roots, while the parent corm remains disease-free.

The diseased vascular tissue varies in color from light to medium brown (Cinnamon buff, and Saccardo's umber in Ridgway (19)). The fungus apparently develops in advance of the discoloration, as it can be isolated from vessels that are still normal in color. The texture of the infected vascular tissue is rather firm, woody, or tough. Under moist conditions the fungus passes beyond the vascular tissues and causes a general, often soft rot of the fleshy part of the corm with various secondary organisms usually present. In plants that have continued growth to or nearly to maturity, the course of the disease is easily traced from the roots through the parent corm into the new corm (Fig. 1, *C*). The exterior of such corms is usually normal. As the tissues dry, small cavities form inside the diseased corms and in these the delicate white hyphae and occasionally the microconidia of the fungus are found.

Microscopic examination of fresh or fixed sections shows fungus hyphae inside the vessels (Fig. 3), abundant in some, rather scanty in others. In the margins of some infected areas the mycelium eluded microscopic obser-

vation, but from similar tissue the fungus could be demonstrated by culturing.

In addition to the typical vascular disease, there is the less typical corm condition variously designated as doughnut, high crown, or hollow core. Beginning at the base there is a progressive browning, drying, and shrinkage

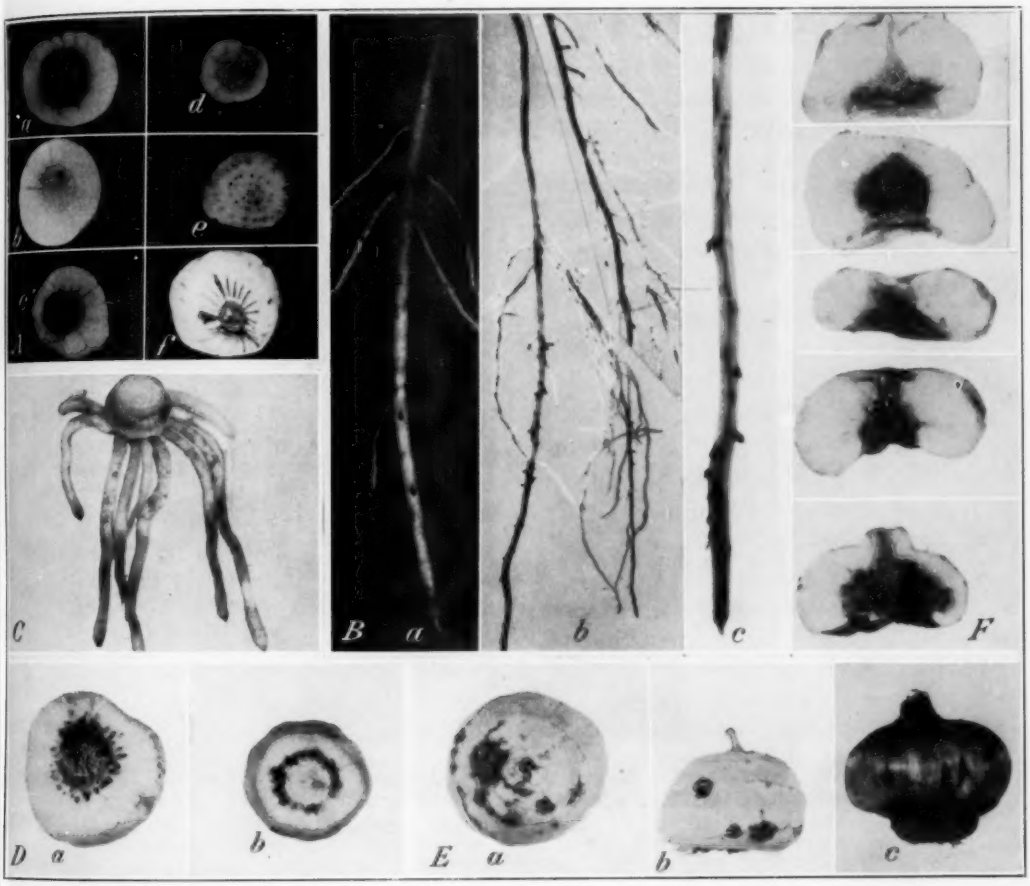

FIG. 2. Vascular infection of gladiolus. *A*. Immature corms: *a–d*, core decay in cross sections; *e*, small lesions along the nodes; *f*, black streaks in husks due to infection. ×½. *B*. Infected fibrous roots. *a* and *b*, grown in sand infested with *Fusarium*; respectively, ×1½ and ×1; *c*, grown in water with *Fusarium* added; the cortex is translucent, showing the dark, infected central vascular strand; ×1½. Note that infected areas are dark. *C*. Infected contractile roots, grown in soil infested with *Fusarium*. ×½. *D*. Base of fibrous roots, blackened by infection: *a*, exterior; *b*, cut across base of corm. *E*. Interior vascular infection breaking through to the surface along the nodes (*a–b*), and extending into the husks (*c*). ×½. *F*. Various stages in the "doughnut" type of rot. ×½.

of the core tissues. Extreme examples have large holes through the center, while the early stages have merely a slight discoloration in the basal scar. Various intermediate stages are found (Fig. 2, *F*). The hole or cavity is usually widest at the base, and in its enlargement may destroy part or all of the root plate. At the top it is ¼ to ½ inch in diameter, sometimes larger,

often including and destroying the terminal buds. The extent of the cavity is limited to the core tissues with occasional slight extensions into the radiating vascular strands. The brown wall of the cavity is thin (½ to 2 mm. thick), hard, and woody. The outer surface of this wall is smooth or occasionally with thorn-like projections into the sound, fleshy part of the corm. The whole of this dry, hard core can be separated easily and cleanly from the rest of the corm.

The doughnut, as well as the vascular, type of infection begins in the field, and the extent of the rot depends on the length of time between infection and harvesting. In very moist soil the corms may rot entirely. After

FIG. 3. Stained section from an infected gladiolus corm. Note three hyphae of the *Fusarium* in longitudinal view and a few in cross section. ×1,370.

digging and curing the central part becomes hard and dry and tends to disintegrate and to fall out, leaving a clean, walled cavity or hole from the bottom to the top of the corm. Corms measured when placed in storage and again in the spring showed little or no enlargement of the cavity during storage at 5° to 10° C.

All of the few plants found with small aerial corms (Fig. 1, *D*) had the doughnut condition in the parent corms below ground. These aerial corms were sound and clean in appearance, but no isolation of the fungus was attempted.

The distribution and occurrence of the doughnut type of disease coincides with that of the vascular type. Whether infection results in the vascular or the doughnut condition seems to depend chiefly on the variety, but possibly, also on the age of the corm, the soil, and the virulence of the parasite. The variety Dr. F. E. Bennett is very subject to the doughnut disease. Less

frequently it occurs in Commander Koehl, Mrs. Francis King, Mrs. Frank Pendleton, Halley, Schwaben, E. J. Shaylor, Virginia, and others that are also susceptible to the vascular infection. High crown to doughnut symptoms have been found in a few corms of Picardy, Giant Nymph, and Golden Dream.

Root Symptoms

In fibrous[2] roots the first indication of disease is a rusty color, becoming darker, even black. The infection may occur at any point along the root, but most frequently at the tip. Lateral roots die back, leaving a dark spot on the main root (Fig. 2, *B, a*). Main roots are invaded at their tips, at the bases of laterals, or at any other point. Unless checked, the fungus continues its growth and enters the root plate.

As new roots continue to push out from the root plate, some may escape infection. When, because of disease or injury, the root plate is destroyed, roots often develop from parts above it, even halfway up the side of the corm.

The infected roots soon die, disappear, or are reduced to thin, wiry strands. The dark vascular bundle of the infected root can be seen through the translucent cortex in roots grown in water or wet sand (Fig. 2, *B, b–c*). The proximal ends of the fibrous roots are embedded in the base of the corm to a depth of 3 to 5 mm. or more. These root bases, normally clean and white, are blackened by the Fusarium infection (Fig. 2, *D*).

When all of the root bases are invaded and darkened the parent corm shows a complete dark ring around the core (Fig. 1, *C, f*). If only a few roots in one part of the root plate are diseased, only a part of the vascular ring of the corm is discolored (Fig. 1, *C, d–e*).

Contractile Roots

Contractile roots[3] (description of fibrous roots applies in general to contractile roots), developing comparatively late in the season, seem less subject to infection than the fibrous roots. These roots become infected at any point along their length but most frequently in the thick upper part and particularly on the under side. Lesions of considerable size often escape observa-

[2] Fibrous roots are the long, slender, freely-branching roots that develop at the base of the parent corm shortly after planting. In damp storage these roots often begin growth before planting. In the soil they persist and function as feeding roots until the new corm is matured.

After the foliage leaves are well grown and the new corm has begun to enlarge at the base of the new shoot, contractile roots develop from the nodes at the extreme base of the new corm. These roots are thick, fleshy, white, and more or less transversely wrinkled. They extend deeply into the soil and have few or no branches. Their function seems to be chiefly that of support or anchorage for the plant. Deep-planted corms are less likely to develop contractile roots than corms nearer the soil surface. Stolons, originating from the same region as the contractile roots, develop somewhat later and produce cormels. The exact point of attachment of contractile roots and stolons is not easily determined because of the extremely short neck or stem between the old and the new corms. However, when the two corms are separated in the cleaning process, the dry, shriveled contractile roots and the stalked cormels are most likely to remain attached to the new corm, at least temporarily, and thus indicate their placement. Further details of gladiolus corm morphology are supplied by Geiger (6).

[3] See footnote 2.

tion until the under side of the root is examined. Infection is first visible as a small spot or red or red-brown streak. From this early stage pure cultures of *Fusarium* can be isolated. The lesions (Fig. 2, *C*) become more or less elongated, ½ to 2½ inches long, often confined to one side of the root, or the root may be girdled. In advanced stages the whole root is brown and shriveled. Dissection and stained sections show that the infection starts in the cortex and penetrates into the vascular bundle, which becomes dark, reddish-brown. Infection spreads in both directions and may extend into the vascular tissue of the new corm.

THE COURSE OF NATURAL INFECTION

New corms can be infected directly from infected parent corms or from the soil. From the soil the *Fusarium* may gain entrance through fibrous roots, through contractile roots, or through the leaf bases near or below the soil line.

When fibrous roots are attacked, the hyphae grow upward in the large straight vessels of the root until they reach and enter the root plate and the vascular tissues of the corm. The vessels of the corm are much convoluted but their general direction is upward. Growing in these large vessels, the fungus eventually penetrates into the new corm, where it often causes more decay than in the parent corm.

From infected contractile roots the fungus grows through the vessels to and into the vascular tissue of the new corm, where infection is indicated by a discolored area at the base of the root. Sections show that the contractile roots, like the fibrous roots, extend a few millimeters into the corm, and that the vascular bundle of the root passes into the corm as a compact cylinder, then spreads out fanlike and merges with the vessels of the new corm. It may happen that only one of two or more daughter corms becomes infected from contractile roots.

If infection has originated from both fibrous and contractile roots, the point of origin of infection is difficult or impossible to determine. Infection of either daughter or parent corms can take place from either fibrous or contractile roots, but perhaps only in severe infection of long duration would the parent corm become infected from a contractile root. Infection continues in the cortex of the contractile root and may possibly reach the new corm by this path, as well as through the vascular tissues, but microscopic examination has failed to prove infection from the cortex. Infection may or may not reach the corm before digging time. It is likely that the infection may continue to spread during curing from the drying root into the more succulent tissues of the new corm.

Another point of infection, particularly in moist weather, seems to be along the stem or compacted leaf bases near or below ground level. Infection in these parts progresses downward through the leaf bases to the nodes of the new corm, where small brown lesions develop. These lesions are like those produced by the extension of infection from the interior of the corm,

except that they lack any connection with interior rot. The node lesions originating from stem infection may be shallow or of considerable depth. Such infections have been observed frequently, but not experimentally produced by the writer. Surface lesions caused by this *Fusarium* develop only from infected vascular tissue of corms or leaves.

The progress of the disease and the appearance of leaf symptoms are determined by the extent of the infection in the planting stock or the degree of infestation in the soil, or both, also on the variety of gladiolus, and the temperature and moisture conditions. Weather conditions have considerable effect on the progress of the disease. Low air and soil temperatures retard the development of the *Fusarium*, while very moist soil and high temperatures accelerate its growth and also favor secondary organisms that hasten the destruction of the corm.

When diseased corms are planted in clean soil they regularly yield a diseased crop or none, and furthermore, contaminate the soil. Corms with very slight infection occasionally outgrow it and produce good plants and clean new corms.

In undisturbed soil the fungus seems not to spread rapidly or far in one season. Susceptible varieties of gladiolus have reached maturity in perfect health when grown 6 inches from others so seriously infected that they died before blooming.

The presence of the fungus can be demonstrated by isolation from any point along the diseased root, at its base in the corm, or in the infected area in the corm. The course of infection is easily followed because of the discoloration caused by the fungus. In both free-hand and stained sections of recently infected roots and corms the hyphae of the *Fusarium* are found only in the tracheae. Some tracheae are full of the strands of hyphae, others show few or none (Fig. 3).

ISOLATION AND PATHOGENICITY OF THE FUSARIUM

Isolation of the Pathogen

Hundreds of isolations during the past 18 years have demonstrated the constant association of a certain type of *Fusarium* with the typical vascular disease of gladiolus. The greater number of these isolations were made from plants grown and experimentally infected in the course of a study of the disease. In addition, isolations were made from infected corms of many varieties that were received from practically all of the gladiolus-growing regions of the United States and a few from Holland.

From recently attacked corms and especially from the developing new corm infected directly from the parent corm, the *Fusarium* is easily isolated in pure culture. In many cases the *Fusarium* was isolated from several parts of the same corm, the core, the radiating vascular bundles, the husks, and from node lesions when any were present.

The pathogen has been isolated from both fibrous and contractile roots and from the base of these roots. It has been isolated from the stem or com-

pacted bases of leaves and flower stalk as much as 6 inches above the ground level. While there are certain very typical symptoms in most cases of this vascular disease of gladiolus, there are also some differences due to the variety, to the stage of infection, and to the conditions of growth or storage. Isolations were made from all such unusual or doubtful specimens.

Under the usual growing or storage conditions the *Fusarium*-infected corms are soon invaded by one or more secondary organisms. Because of the presence of these secondary organisms, the isolation of the causal *Fusarium* from advanced stages of the doughnut type, especially from the cured, stored corms, is difficult.

Every effort was made in isolations and transfers to secure and maintain only pure cultures and nothing has developed to cast any doubt on the purity of the majority of the isolates. However, to satisfy the demand for data obtained from single spore cultures, many such isolations have been made. Spores from a culture were dispersed in water and diluted until there were only 10 or 12 spores in a large drop. Such a dilution was spread over the surface of a hard-agar plate. With only 5 cc. of clear agar in a plate, the spores are easily seen in microscopic examination through the bottom of the plate. When the spores germinate they are cut out, placed on a slide, and again examined before transfer to a tube as a single spore. For most isolates, with few or no macroconidia, microconidia were used for the single-spore isolations. Several single-spore isolates from each of 22 isolates were checked for pathogenicity and cultural and morphological characters.

Methods of Inoculation

The most reliable and natural method of inoculation is to plant corms in sterilized soil or sand to which the pathogen has been added at, or shortly before, planting time.

The *Fusarium* for inoculation purposes was usually grown on steamed oats, sometimes on rice, potato, or other media. When well developed the growth was finely divided and measured amounts thoroughly mixed with the soil in the lower part of the pots or bench. The corms were placed on this inoculated soil and covered with several inches of clean soil.

In none of the inoculation experiments did the *Fusarium* affect the intact epidermis of corms, even when the inoculum was placed directly on the corm. Some wounded areas developed decay varying from small, shallow brown spots to rather extensive rot, sometimes reaching and entering the vascular tissues.

When Fusarium cultures, crushed and diluted in water, were poured over plants nearing maturity, the fibrous roots and the bases of the parent corms became infected, but the disease spread into very few of the new corms. Possibly older plants are somewhat resistant, or the fungus did not have time to invade all parts of the plants. In comparable trials on the same varieties of gladiolus, inoculum added to soil at planting time resulted in 85 to 100 per cent infection, while that added to soil 8 weeks after planting produced only 40 to 70 per cent infection.

To observe the effect of inoculation on roots, corms were grown over sterile tap water. In sterile water the roots remained clean and free from disease but those in water containing *Fusarium* soon discolored and rotted (Fig. 2, *B, c*). On isolation from such roots, *Fusarium* and often also bacteria and *Penicillium* were recovered. Clean, sterile, wet sand proved a good medium for root inoculation experiments, as frequent examinations could be made without injury to the roots.

Mature leaves of gladiolus were inoculated in several tests. When the inoculated area was kept moist, a general rot with only slight indication of vascular infection developed in which *Fusarium, Penicillium,* and bacteria were found. If the inoculated area was exposed to the usual atmospheric conditions, no infection resulted.

While a large number of varieties have been used in inoculation tests, those most frequently used were Mrs. Francis King, Mrs. Frank Pendleton, Dr. F. E. Bennett, Mrs. Dr. Norton, Tyco Zang, E. J. Shaylor, Crimson Glow, Schwaben, Alice Tiplady, Giant Nymph, and Tyrian Beauty. It was only by chance that most of the early tests were made with susceptible varieties.

Pathogenicity

In the beginning of this study an effort was made to test the pathogenicity of each organism isolated from corms received for examination and diagnosis. When, after numerous tests, a certain type of *Fusarium* was recognized as the responsible pathogen, the tests were restricted to isolates from different varieties of gladiolus, from different localities, or from specimens showing atypical symptoms.

For the pathogenicity tests, selected corms were carefully examined for freedom from disease and grown under conditions that precluded infection from outside sources. Also a large number of control plants were grown, thus reducing the chance of error from incipient corm infection.

The inoculations were made by adding the Fusarium cultures to the soil in which the corms were planted. The plants, if susceptible, developed a typical infection, while the adjacent control plants remained healthy. From various parts of the infected plants the *Fusarium* was reisolated and these reisolations, when compared with each other and with the original isolate, were found to agree in cultural and morphological characters. Also, the reisolations were tested by inoculating plants and proved pathogenic.

In a field trial (detailed in the section on varietal susceptibility, p. 284), 18 isolates of the vascular *Fusarium* were tested separately on each of 20 varieties of gladiolus. From the 7 more susceptible varieties reisolations were made from infected vascular tissue of the new corms; these have been compared with the cultures used as inoculum by cultural tests on 5 different media (potato-dextrose, corn-meal, corn-meal-plus-dextrose, and oatmeal agar, and bean pods), and by spore measurements. Judged by these comparisons, the Fusarium isolates recovered from the infected gladiolus are of the same type as those used to inoculate the soil.

Experimental proof was obtained that contractile roots are infected by the vascular *Fusarium* and that they transmit the disease to the new corms. In these tests corms of 2 varieties, Dr. F. E. Bennett and Chas. Dickens, 30 corms of each, were grown singly in sterilized soil in 6-inch pots. The inoculum was applied after the contractile roots were well developed. The surface soil was removed carefully to minimize wounding of roots, a Fusarium culture in a water suspension was poured over these, and the soil replaced. With this method a number of contractile roots became infected and all infection in the new corms could be traced to infected contractile roots. The *Fusarium* was reisolated from a number of contractile roots and corms, and pathogenicity of several of the reisolates was demonstrated. No infections developed in the 10 control plants of each variety.

Observations and experiments indicate that the pathogenicity of isolates from "doughnut" types of infection is less than that of isolates from the typical vascular types. Inoculation with the "doughnut" isolates produced typical vascular infection as often as they did the doughnut type, but infection was slower and less extensive than in parallel inoculations with the vascular isolates.

That pathogenicity of this vascular *Fusarium* is reduced by long periods of culture in artificial media has been generally noted but no extensive records have been kept. Three isolates, Nos. 30, 32, 34, of 1935, tested shortly after isolation, were unusually virulent, causing 100 per cent infection in the varieties Panama, America, and Mrs. Francis King. In the same experiment with the same varieties, four older isolates (1929, 1930, 1933) produced 40, 62, 53, and 68 per cent infections, respectively. In 1936 the isolates of 1935 again gave 100 per cent infection on the varieties Dr. F. E. Bennett, Crimson Glow, and Schwaben. In 1938 the variety Dr. F. E. Bennett was inoculated with No. 34 of the 1935 isolates and No. 14 of the 1929 isolates. Isolate No. 34 caused only moderate infection and isolate No. 14 a mere trace. In 1939 isolate No. 34, used in an experiment for observation of root infection, caused only traces of disease, while younger isolates were definitely virulent. Factors other than age may be partly responsible for such decline in virulence. This change has been observed so frequently that cultures 2 years old or younger have been used in all recent experimental work. In addition to such loss of virulence there are even among recent isolates differences in degree of pathogenicity. Some infect a wide range of varieties of gladiolus, others infect only the most susceptible varieties, and some isolates from typical lesions produce no infections.

CHARACTERISTICS OF THE FUSARIUM

Comparison of Isolates

Beginning in 1923, isolates from the yellows disease have been compared and records made of their characteristics and particularly of their pathogenicity. None of the earlier isolates are now in stock but the records and the camera-lucida drawings of these agree perfectly with those of the past

5 or 6 years. The *Fusarium* cultured from gladiolus of various geographic origins, from many different varieties, and from the several plant parts including old and new corms, fibrous and contractile roots, and leaf bases, have been compared. Also reisolations have been checked against original isolations and single-spore cultures against source cultures. The various isolates are very similar in physiology and morphology, although the character of growth of even a single spore isolate may vary considerably with slight differences in the medium or the environment. Variation in *Fusarium* is an old story and is noted in practically all studies of species of this genus. In 1904 Smith and Swingle (21) wrote of *Fusarium oxysporum:* "Judged by the above descriptions we have had half a dozen species of *Fusarium,* yet all were the product of a single spore."

Most isolates have been similar or identical in appearance on the usual culture media. Sectoring has not been observed in either plate or tube cultures. Differences sometimes occurred that suggested separation of the isolates into several groups, but on repetition of the tests these differences proved inconstant. The isolates from various localities and from different parts of the gladiolus plant have proved, on the whole, strikingly similar in the amount and character of growth and pigment. The only apparently constant cultural distinction noted is a difference in aerial growth in Tochinai (24) agar.[4] On this medium some isolates have a fairly persistent surface growth so dense that the color of the stroma does not show through, while in others the aerial growth usually collapses into a flat, appressed layer much the color of the stroma.

Cultures have shown perhaps more variation in pathogenicity than in cultural character. Of 18 typical, single-spore, young isolates (from pathogenic stock cultures) used to inoculate 10 varieties of gladiolus (known to be susceptible) only 1 (12–1) of the 18 produced infection in all of 10 varieties. Three isolates infected 9 varieties; 9 isolates infected 8 varieties; 3 isolates infected 7 varieties, and 2 isolates infected only 6 varieties.

Comparison with Fusarium oxysporum var. gladioli Massey

In addition to the wilt or yellows-producing *Fusarium* of gladiolus, it is to be noted that *Fusarium oxysporum* var. *gladioli,* described by Massey in 1926 (8), is the cause of a gladiolus disease. A culture of this organism, received from L. M. Massey, sub-cultures and reisolations from it, and isolates made by the writer from typically diseased plants, have been included with the vascular pathogen in parallel tests of cultural and morphological characters and pathogenicity. Five cultures of *F. oxysporum* Schlecht. have also been compared with the two pathogens from gladiolus. Four of these cultures were obtained from Freeman Weiss, the fifth from Miss Lillian Cash. In culture the bulb-rot organism has, in most tests and examinations, shown less abundant aerial growth, less pigment, and wider macrospores than the yellows organism. The mycelium of the former also shows a ten-

[4] Modified Tochinai Agar: Peptone, 0.5 g.; Mono potassium phosphate, 0.5 g.; Magnesium sulphate, 0.5 g.; Maltose, 15.0 g.; Agar, 12.0 g.; Water, 1000.0 cc.

dency to be somewhat coarse and to become woolly with age, while that of the yellows pathogen collapses but remains fine in texture. The most distinctive characteristics of these two Fusaria of gladiolus are the effects on the host.

The yellows Fusarium causes a vascular disease of the growing plant, attacking roots and corms early in their development, seriously infecting and even destroying the plants before maturity. Only in late stages of the disease and under moist conditions is the fleshy tissue of the corm attacked and, even then, it is a question whether this *Fusarium* is chiefly responsible for these late stages of rot, as by this time various secondary organisms have invaded the affected corm.

Fusarium oxysporum var. *gladioli,* on the contrary, attacks the surface and causes a rot of the starchy storage tissues. The lesions originate during the growing period, or perhaps during or after harevsting, and continue to develop during storage. Massey (8) says of *F. oxysporum* var. *gladioli,* "It is primarily a storage disease."

In susceptible varieties of gladiolus grown in soil heavily infested with *Fusarium oxysporum* var. *gladioli,* an occasional slight vascular infection has resulted that would be overlooked on casual examination. Such infections have occurred in several inoculation experiments under conditions that precluded infection from sources other than the inoculum placed in the soil. In parallel inoculations the vascular types of *Fusarium* gave 100 per cent severe infections.

The ability of the 2 organisms to cause exterior lesions on corms was compared by inoculating, with and without needle prick wounds, the surface of 5 varieties of mature corms. The corms were kept slightly moist, at 24° C. for 3 weeks. No infection developed, except through wounds. *Fusarium oxysporum* var. *gladioli,* infected all 5 varieties, producing 46 lesions of moderate size, while the vascular *Fusarium,* with an equal number of wounds, infected only 2 varieties and produced only 10 very small lesions.

Cultural Characters

Cultures have been kept at room temperature (22° to 25° C.) and in moderately subdued light, except in a few special experiments.

In most media there is a fairly abundant mycelial growth, white, fine textured, with little or no pigment, except in media containing sugar or acid. With age the aerial mycelium partly collapses and becomes tinged with buff or cream color. On corn-meal agar, both with and without sugar, and on stems of clover and potato, the aerial growth is scanty. Growth is abundant and strongly pigmented (Spinel-red to dull-purple (19)) on rice. No odor has been detected in rice cultures.

On Tochinai[5] agar, the yellows *Fusarium* makes a vigorous growth and produces abundant and vivid colors. The color begins as pale-pink, changes to red (Spinel-red), or reddish-orange (Carnelian-red), then Vinaceous-

[5] See footnote 4.

purple or Hyssop-violet with numerous intermediate tones and shades of these colors. In well-grown plate and slanted-tube cultures the reverse shows a wide marginal zone of radiating lines of various shades of red, red-orange, or purple. When first used this seemed a useful medium for separation of strains because of the variety of colors produced, but isolates grouped according to color often changed rapidly, requiring regrouping in less than a day. In the end they were all practically alike as to color. As stated (p. 284), the isolates seem to fall into two groups in regard to the character of the aerial growth in Tochinai agar.

Microconidia are abundant in all media; macroconidia absent, few, or irregularly produced; pionnotes, very rare; sclerotia, not infrequent in potato agars, small, dark-gray to dark bluish-green; chlamydospores, abundant in all media. Many kinds of media have been used in the effort to increase the production of macroconidia. Oatmeal hard agar and clover stems have given the best results, but even with these media many cultures failed to produce macroconidia. (Three cultures on clover stem and two on bean stem developed pionnotes or sporodochia.)

Morphologic Characters

From a number of recent single-spore isolates of proved pathogenicity, several (7–3, 11–2, 12–1, and 16–1) were selected in 1940 for the morphological description. These were chosen as representing the slight differences in growth and because they produced a sufficient number of macroconidia for study. They were isolated in 1938 from typically infected vascular tissue of gladiolus. Their pathogenicity was tested and single-spore isolates made in 1939. The pathogenicity of the single-spore isolates was confirmed in 1939 and again in 1940, and the *Fusarium* was again reisolated. In gross and microscopical morphology and in physiology these recent isolates and reisolates are very similar to or identical with other recent isolates and also with the numerous isolates of the past 18 years.

Of the selected isolates, No. 11–2 is representative of the group producing persistent aerial growth and less pigment than the majority of the isolates. Number 16–1 most nearly represents the group with aerial growth that frequently collapses in Tochinai agar. The others are intermediate between these two or variable in this respect. In the latest pathogenicity tests, isolate No. 12–1 ranked highest, with an average of 64.5 per cent infection in 10 varieties of gladiolus; No. 11–2 gave 52.5 per cent infection; No. 7–3, 50.5 per cent, and No. 16–1, 49.5 per cent in the same 10 varieties of gladiolus.

The aerial mycelium is white, abundant, and 3 to 5 mm. deep, with age slightly buff. Sub-stratum, colorless to pale-buff, lilac, Spinel-red, or vinaceous-lilac to dark-vinaceous, depending on the medium.

Sclerotia occur occasionally on potato-dextrose agar. They are small, more or less globose, single or clustered, dark-gray to bluish-green or dark-blue, formed in one or two rows in the lower margin of slant agar cultures.

Chlamydospores develop in all media. They are hyaline; usually vacuolate, spherical and 1-celled (when 2-celled somewhat elongated or pear-shaped); terminal and intercalary

in the mycelium and in the macrospores; mostly single but sometimes in chains of three. The walls are smooth (rarely in very old cultures somewhat irregular or rough), mostly thin, becoming thicker with age. The 1-celled are 7 to 10 μ in diameter, the 2-celled 12 to 15 μ.

Microconidia are abundant in all isolates: hyaline; continuous or 1-septate; 90 per cent ovoid, a few kidney-shaped or ovate (Fig. 4). The size ranges from 3 to 14 × 2.2 to 5.0 μ, but the majority are 7 to 10 × 2.8 to 4.2 μ.

Macroconidia are scarce, often lacking. In one culture tube (not a single cell isolate) a few *sporodochia* were found. *Pionnotes* are rare and, as stated earlier, have been observed in only three culture tubes (clover stem, bean stem, corn-meal agar). These three were the only cultures found with a large number of macroconidia. In most cases the macroconidia are scattered in the aerial mycelium along with the microconidia. Even when macroconidia were reasonably numerous, their size, especially the diameter and the septation, varied not only in the different media, but also at different ages in the same medium. As an example, parallel cultures of isolate No. 16–1 were made in oatmeal agar, Tochinai agar, potato-dextrose agar, corn-meal agar, and rice. In oatmeal and Tochinai agars the macrospores were 3.5 to 5.6 μ in diameter, while at the same age (24 days) the spores from rice, corn-meal, and potato-dextrose agar were only 3.0 to 4.5 μ in diameter. Potato-dextrose-agar cultures 10 days old had macrospores 3.0 to 4.4 μ in diameter. Ten days later, spores from the same tube were 4.2 to 4.9 μ in diameter.

The macroconidia seem not to remain long in what is considered a normal condition. If too young they do not have the width of mature spores and the septation is poorly developed. When too old the spores are either shriveled or swollen (depending on the moisture content of the medium), hyaline, full of vacuoles, often with broken or absorbed apical cells, or with chlamydospores in the cells or at the ends. Such changes have been noted in all of the isolates. If macroconidia are produced at all they reach a stage of normal development and then rather rapidly deteriorate, even disintegrate. The normal, mature macroconidia in good condition are homogeneous or finely granular, with walls and septa well developed, and cells neither swollen nor shrunken. They are mostly straight, yet many are slightly curved. The central part is nearly straight and of equal diameter; the ends are pointed, the base with a pedicel more or less developed, and the apex curved or bent (Fig. 4).

Because of the scarcity of macroconidia it was decided to try to find and measure 50 of the 3-septate spores from as many isolates as possible and all spores with other septation found during the search for the 50. In many cases it was necessary to search a number of mounts to secure the desired number. In those unusual mounts in which macroconidia were relatively numerous, 100 of the 3-septate forms were measured. The 4- and 5-septate spores are rare and only one 6-septate spore has been seen.

For measuring, the spores were mounted in water on a thin layer of clear agar and the cover sealed with vaseline. The slides were prepared by flooding them with melted water agar, drained quickly, and dried. When the spores, in a drop of water, are mounted on such a slide the agar film softens sufficiently to hold the spores in place and in one plane. The spores were measured immediately after mounting. Such slides if protected from dust remain in good condition for days.

An attempt was made to have the spores to be measured of the same age, grown in the same medium and under the same conditions. This was not entirely successful, as the development of spores was variable, even under

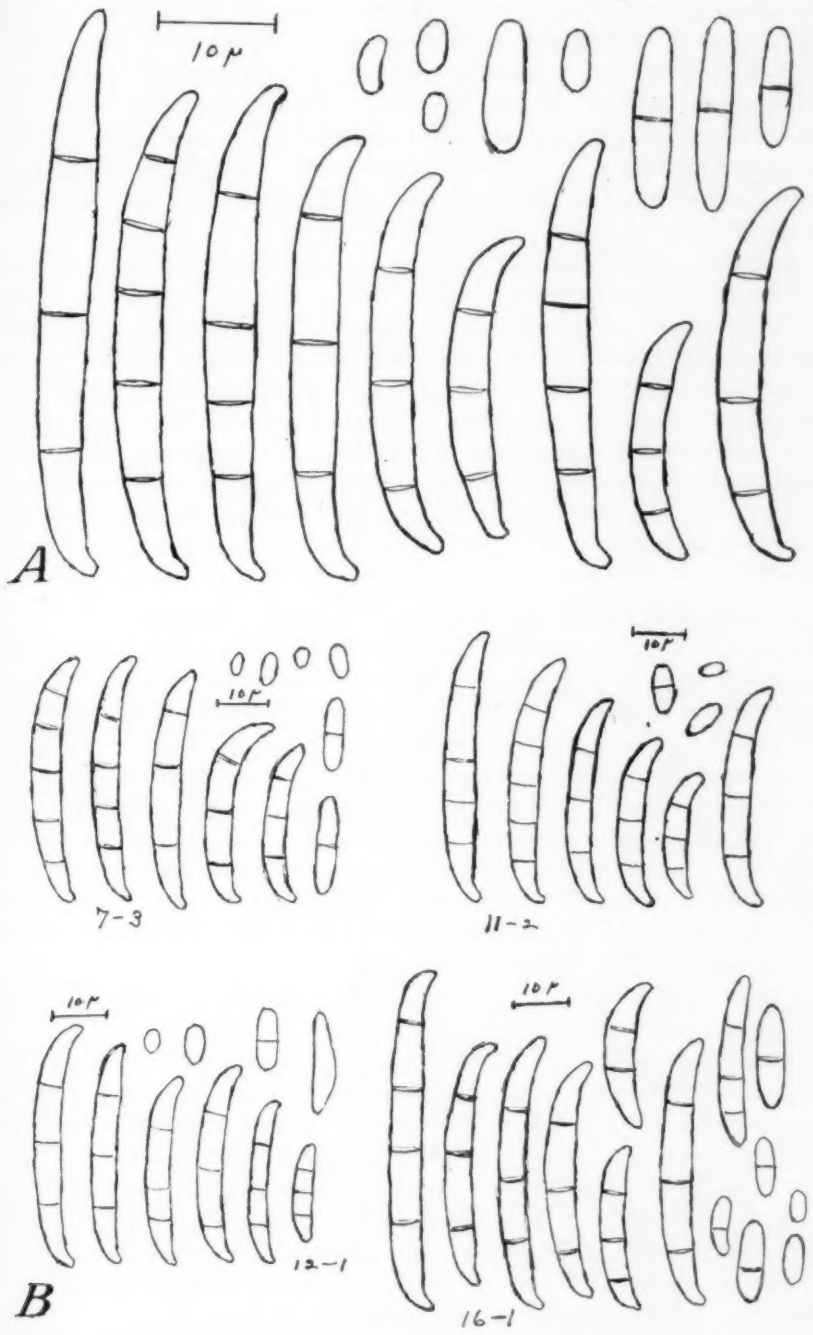

FIG. 4. *A*. Spores of *Fusarium orthoceras* var. *gladioli* from potato-dextrose-agar culture. *B*. Spores of *F. orthoceras* var. *gladioli*, produced by single-spore cultures of isolates 7–3, 11–2, 12–1, and 16–1, respectively. (See table 1 for media.)

apparently identical conditions. In some cultures, normal macroconidia were present in 14 to 18 days, in others, not until considerably later. Isolate No. 7–3, when 20 to 30 days old, had normal 3-septate macroconidia, and 6 to 9 per cent of 4- and 5-septate spores. When 45 days old, the 3-septate spores were scarce and in poor condition and none of the 4-septate or 5-septate type were found. Just the reverse occurred with No. 7–3B in the same medium, where the 3-, 4-, and 5-septate spores were normal and more numerous at 45 days.

Measurements of macroconidia from 4 representative single-spore isolates are given in table 1.

Regardless of some variations in morphology, physiology, and degree of pathogenicity, these isolates from gladiolus are closely similar and have enough stable characters to indicate that they belong to one species in the sub-section Orthoceras. It seems legitimate to establish this *Fusarium* as a new variety on its known host relationship. The apparent restriction to gladiolus (including probably some related plants, as is indicated in the section entitled Other Potential Hosts), seems sufficient reason for describing it as a new variety.

Classification

Because of its morphological and physiological characters it is fairly easy to determine that this vascular pathogen of gladiolus belongs, according to the classification of Wollenweber (26) and Wollenweber and Reinking (27), in the section Elegans. It is somewhat more difficult to decide between the sub-sections Orthoceras and Oxysporum. The rare production of pionnotes, the few sclerotia, an occasional culture with numerous macroconidia, and the fact that *Fusarium oxysporum* var. *gladioli* is able to cause a slight vascular infection in gladiolus, suggested a relationship with the sub-section Oxysporum and possible identity with *F. oxysporum* var. *gladioli*. But these similarities are not sufficient to balance the entirely different disease symptoms produced in the host by the vascular pathogen, the general nonproduction of sporodochia, pionnotes, or sclerotia, and the more slender and straighter macroconidia, all of which indicate a closer relationship to the Orthoceras than to the Oxysporum sub-section.

There is not sufficient discrepancy in the physiological or morphological characters of the four selected representatives or among the hundreds of other isolates from gladiolus to warrant further separation within the variety. In certain respects the variations found are considered no greater than those occurring in many if not most species and varieties of *Fusarium*.

An important distinction of the vascular parasite of gladiolus is its host relationship, which seems sufficiently important to establish it as a variety of the type species of the sub-section. The name *Fusarium orthoceras* var. **gladioli** n. var. is proposed for this pathogen.

Technical Description

Fusarium orthoceras App. et Wr. var. **gladioli** new var.

Mycelium typically aerial, white, abundant, fine-textured; on corn-meal agar appressed, colorless. Color of substratum in beef-agar cultures buff to chamois; in oatmeal

TABLE 1.—*The range and average size in microns of the macroconidia of 4 representative cultures of Fusarium orthoceras var. gladioli isolated from gladiolus in 1938. Single-spore isolates in 1939. Measurements made 1939–40*

Isolate number	Source of isolate	Age of culture	Macroconidia measured		Measurements	
					Range	Average
			Septations	*Number*	*Microns*	*Microns*
7–3	Husk tissue of stored corm (variety Blue Boy) grown in Michigan 1937.	30 days on oatmeal agar	0	0	.....	.....
			1	9	15.5–19.6 × 3.5–4.2	.....
			2	2	16.8–19.6 × 3.5	.....
			3	100	16.8–47.6 × 3.0–4.8	31.8 × 4.2
			4	9	30.0–45.5 × 4.2–4.5	.....
			5	6	36.4–40.6 × 4.2–4.5	.....
			6	0	.....	.....
11–2	Vascular tissue of new corm (variety Mrs. Frank Pendleton) grown in soil inoculated with vascular *Fusarium*, isolate 11.	34 days on oatmeal agar	0	0	.....	.....
			1	2	14.0–15.5 × 3.5	.....
			2	1	19.6 × 4.0	.....
			3	50	16.8–49.0 × 2.8–5.6	30.8 × 4.2
			4	2	32.2–36.5 × 3.5–4.2	.....
			5	1	25.5 × 4.3	.....
			6	0	.....	.....
12–1	Vascular tissue of new corm (variety Mrs. Francis King) grown in soil inoculated with vascular *Fusarium* 12.	35 days on potato dextrose agar	0	0	.....	.....
			1	3	18.0–22.0 × 3.0–4.4	.....
			2	1	19.6 × 4.2	.....
			3	50	18.2–37.8 × 3.5–4.3	28.3 × 3.8
			4	0	.....	.....
			5	0	.....	.....
			6	0	.....	.....
16–1	Vascular tissue of new corm (variety Mrs. Francis King) grown in soil inoculated with the vascular *Fusarium* 16.	24 days on oatmeal agar	0	0	.....	.....
			1	5	25.0–25.5 × 4.2	.....
			2	0	.....	.....
			3	100	22.5–54.6 × 3.5–5.6	35.7 × 4.3
			4	1	53.2 × 4.4	.....
			5	0	.....	.....
			6	0	.....	.....

agar pale-pink to Spinel-red and vinaceous-purple;[6] in rice, aerial mycelium white, submerged hyphae and rice pale-pink to Spinel-red, Jasper-red and dark, dull-purple; no odor from rice cultures. Microconidia abundant, mostly non-septate, 90 per cent ovoid, 3–14 × 2.2–5 μ, mostly 7–10 × 2.8–4.2 μ; the few 1-septate, 14–2.5 × 3.0–5 μ. Macroconidia typically scarce; scattered in the aerial mycelium, very rarely in pionnotes; straight to slightly curved; slightly pedicellate, moderately dorsiventral; ends pointed and curved; typically 3-septate, 31.5 × 4.2 range (16.8–54.6 × 2.5–5.6); 4-septate, rare, 40.9 × 4.7 (range 30.0–53.0 × 3.5–5.6); 5-septate, rare, 41.5 × 4.7 (range 25.5–54 × 4.2–5.6); the 0-, 1-, and 2-septate macroconidia, rare in mature cultures, 10–40 × 2.5–4.5, average diameter 3.6. Sporodochia none; pionnotes very rare; sclerotia occasional. Chlamydospores abundant in mycelium and macroconidia; terminal and intercalary; spherical to oval; single and in short chains; smooth; single, 7–10 μ, 2-celled, 12–15 μ. Optimum temperature 23° to 26° C. Parasitic in tracheae of fibrous and contractile roots and corms of gladiolus; cause of Fusarium yellows in horticultural varieties of gladiolus, of the Nanus, Primulinus, and Grandiflorus forms.

TEMPERATURE RELATIONS

Effect of Temperature on Growth of Fusarium in Culture

In slanted tubes of potato-dextrose agar sealed to prevent evaporation, the minimum temperature for growth of the vascular *Fusarium* is below 3° C., a trace of growth becomes visible in 4 days at 3° C. The optimum lies in the range of 23 to 26° C. and the maximum at about 34 to 36° C. At 34° C., growth visible with a hand lens increases slowly but remains scanty and restricted to the inoculated area. No apparent growth occurs in 2 weeks at 36 and 37° C., but when the tubes are removed to 24° C., normal and abundant growth develops in 5 days.

Freshly inoculated potato-dextrose tubes kept at −20° C. for 35 days, then removed to 24° C., developed visible growth in 3 days and the slants were covered by normal growth in 6 days.

The effect of soil temperature was determined by growing gladiolus in metal cans suspended in Wisconsin type tanks of water. The variation in the water temperature, maintained and controlled electrically, was slight, not more than 1 to 2° C. The temperatures ranged from 15 to 32° C. A suitable moisture content of the steam-sterilized soil was kept constant. Cultures of the *Fusarium* were added to the soil below the corms. To retard evaporation, peat or granulated cork was placed on the soil surface.

Infection of roots and parent corms occurred throughout the range of soil temperatures to which these structures were exposed. Plant growth was best at 15 to 18° C., and, at these temperatures, the new corms often escaped infection. Both the number of plants infected and the degree of infection increased up to 22 to 25° C. Soil temperatures above 25° C. are evidently unfavorable for both the gladiolus and the pathogen, as infections were practically limited to the roots and the base of parent corms.

Three experiments in 3 different years, including 7 susceptible varieties of gladiolus, indicate that the optimum soil temperature for infection is 22 to 25° C. (Table 2). For the records in this table, every trace of infection, regardless of its extent, was regarded as positive. This does not give a true picture as 100 per cent infection at 15° or 18° C. usually represented a much slighter degree of infection than 100 per cent at 22° to 25° C.

[6] Plate 44 of Ridgway's Color Chart. There is another vinaceous purple on plate 38.

TABLE 2.—*Effect of soil temperature on incidence of vascular disease in gladiolus grown in*

TABLE 2.—*Effect of soil temperature on incidence of vascular disease in gladiolus grown in soil inoculated with Fusarium orthoceras var. gladioli, F. oxysporum var. gladioli, or Trichoderma sp.*

Soil temp.	1932 experiment[a] Infection in parent corms and new corms inoculated with					
	F. orthoceras var. *gladioli*		*F. oxysporum* var. *gladioli*		Uninoculated controls	
	Parent corms	New corms	Parent corms	New corms	Parent corms	New corms
°C.	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
15	75	38	0	0	0	0
18	60	25	0	0	0	0
21	45	50	0	0	0	0
24	44	70	0	0	0	0
28	43	(*)	0	(*)	0	(*)
32	15	(*)	0	(*)	0	(*)

	1934 experiment[b] Infection in new corms inoculated with					
	F. orthoceras var. *gladioli*			*F. oxysporum* var. *gladioli*	*Trichoderma* sp.	Uninoculated controls
	Isolate					
	#1	#2	#3			
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
15–16	66.6	83.0	0	0	0	0
18–19	81.2	12.5	0	0	0	0
24	100.0	50.0	50.0	0	0	0
28	(*)	(*)	(*)	(*)	(*)	(*)

	1936 experiment[c] Infection in new corms inoculated with *F. orthoceras* var. *gladioli*							
	Isolate							Uninoculated controls
	#13	#14	#15	#23	#30	#32	#34	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
16	50	25	0	50	75	75	75	0
19–20	0	0	25	0	100	100	50	0
26–27	0	25	0	75	50	75	100	0

(*) No new corms developed.

[a] Test varieties, Mrs. Francis King, Schwaben, Tyrian Beauty.

[b] Test varieties, Mrs. Francis King, Mrs. Frank Pendleton, and one unknown variety.

[c] Test varieties, Dr. F. E. Bennett, Schwaben, Crimson Glow.

In experiment 3 all of the parent corms in the inoculated lot were infected. In contrast all of the parent corms in the control lot remained free from disease.

In addition to the noninoculated controls in each experiment, *Fusarium oxysporum* var. *gladioli* and *Trichoderma* sp. were used to inoculate the soil and were carried at the same temperatures as the vascular *Fusarium*. No infections resulted from these, and the plants developed normally and exactly like the controls.

VARIETAL SUSCEPTIBILITY

Among the comparatively few varieties of gladiolus used in experiments, differences in reaction to the vascular parasite ranged from high resistance to complete susceptibility. In parallel experiments in heavily infested soil and with conditions favorable for the parasite, the susceptible varieties developed extensive infection and died before blooming. The highly resistant varieties were only occasionally and slightly infected (2 to 4 per cent) and usually without any foliage symptoms, the infection being discovered only by cutting the corms.

Both field and greenhouse tests have demonstrated (12) that the following varieties of gladiolus can be listed as resistant: Albania, Alice Tiplady, Apricot Glow, Dearborn, Giant Nymph, Hopi, Los Angeles, Minuet, W. H. Phipps, Picardy, Souvenir, and Spirit of St. Louis.

The following varieties have been found susceptible: America, Anna Eberius, Ave Maria, Bloomington Beauty, Blue Boy, Byron L. Smith, Charles Dickens*, Chicago White, Commander Koehl, Crimson Glow*, Dr. F. E. Bennett*, Emile Aubrun, Edith Mason, Evelyn Kirtland*, Flaming Sword, Golden Measure*, Halley, Herada, Kalamazoo, Lansing, Le Marechal Foch, Maidens Blush*, Marie Kunderd, Mary Shary, Mrs. F. L. Karcher*, Mrs. Francis King*, Mrs. Frank Pendleton, Niagara, Odin, Orange Queen, Panama, Pride of Goshen, Pride of Wanakah, Purple Glory, 1910 Rose, Schwaben, E. J. Shaylor*, Tempa, Tyrian Beauty, Virginia*, War*. Varieties starred above have proved very susceptible in our tests; leaf yellowing and wilt occur almost as soon as the leaves appear and the whole plant is usually dead before flower buds form. The other varieties show foliage symptoms later in the season and the new corms may escape infection if growing conditions are favorable.

It is interesting to note that the resistant variety Picardy is the off-spring of a cross between Apricot Glow, a resistant variety, and Emile Aubrun, which is of intermediate rank in the writer's experiments. It, therefore, seems possible to secure resistance in a new variety even when one parent is more or less susceptible.

Other Potential Hosts

In parallel inoculation experiments on growing gladiolus, freesia (*Freesia* sp.), potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), and celery (*Apium graveolens* L.), typical vascular infections were produced in the gladiolus, but no trace of infection of any sort in potato, tomato, or celery, and no definite infection in the freesias. Inoculation tests on other hosts might add to the list of host plants. At present, the

writer has found this *Fusarium* in *Gladiolus*, *Tigridia*, and *Montbretia* (*Tritonia*), but has proved its pathogenicity only to gladiolus.

In 1923 freesia corms from California showed disease symptoms similar to those of the vascular disease of gladiolus, and, when planted, behaved exactly as the infected gladiolus. The *Fusarium* isolated from the freesias appeared identical in morphological and cultural characters with the gladiolus *Fusarium* but cross-inoculation experiments made in 1923–24 were not successful. Unfortunately, the variety of gladiolus used in these experiments was not recorded. The freesias used were of the variety Purity. It could be that the failure to infect was due to resistant varieties. Taubenhaus and Ezekiel (22, 23) have described a Fusarium wilt and corm rot of freesias. They failed to secure typical infection of gladiolus on inoculation with the *Fusarium* from freesia. Here again the gladiolus used may have been resistant.

In 1927 the writer isolated from Montbretia and Tigridia corms showing brown vascular rot, a *Fusarium* morphologically and physiologically like that from gladiolus. This fungus proved pathogenic to gladiolus, producing the same vascular type of infection as the *Fusarium* isolated from gladiolus. As test plants were not available, no pathogenicity tests were made on *Montbretia* or *Tigridia*. Crocus corms (*Crocus* sp.) with typical vascular infection have been examined by the writer, but no isolations were made. Drayton (5) has reported a vascular Fusarium disease of crocus.

CONTROL

It is difficult to control a parasite that penetrates so deeply into plant tissues that it cannot be reached by disinfectants or destroyed by temperatures not injurious to the host. Prolonged high or low temperatures, with or without moisture in the soil or in cultures, have had little or no effect in reducing the vitality of the parasite. Hot water treatments, employing various combinations of temperature and time of exposure were useless because the parasite proved more resistant than the host.

Numerous combinations of soil types with soil temperature, soil moisture, soil pH values, and fertilizers have been tried without success in securing clean crops from infested soil or infected planting stock. As a soil disinfectant, chloropicrin (10) was effective in destroying the *Fusarium*. In the chloropicrin-treated plots, even the susceptible gladiolus varieties grew to maturity in perfect health, while the same varieties in adjoining, untreated plots, developed infection and died before blooming.

Infected corms should be disposed of in a manner to prevent spreading the disease. Planting stock should be disinfected to destroy surface organisms. A 5-minute dip in mercurous chloride (Hg_2Cl_2) 3 to 5 ounces in 1 gallon of water is the most efficient disinfectant tried by the writer. Mercuric chloride ($HgCl_2$) 1–1000 for 6 to 8 hours is very efficient. With corms of the "doughnut" type of infection, experiments indicate that disinfection before planting results in a degree of control not attained in the usual vascular type.

As this parasite is now widely distributed and persistent in the soil and soil disinfection is too costly for large-scale use, the selection and use of resistant varieties of gladiolus appear to be the surest method of avoiding the disease. A number of resistant varieties are available (see list, p. 284) covering a wide range of colors and forms. By concentrating on these for breeding, a larger number of resistant varieties should be obtained.

SUMMARY

A disease of gladiolus, widespread and destructive in the United States, is caused by a *Fusarium* that attacks primarily the vascular tissues of the plant. It causes premature yellowing and death of the leaves, and eventual destruction of the corm.

This disease was first observed by the writer in 1923, in corms grown on the Pacific Coast from stock originally imported from Europe. From the West the disease seems to have spread rather rapidly eastward, probably largely by distribution of infected planting stock that, in external appearance, was free from disease.

The fungus enters the roots and the base of the corm and advances upward through the core, browning and destroying the vascular tissues. Many badly infected corms have no exterior sign of disease as only in the late stages of infection are discoloration and rot apparent on the surface of the corm.

The source of the infection may be either infected planting stock or infested soil. Infected planting stock produces poor growth and a poor crop or none; the disease is transmitted directly from the infected parent corms to any new corms that may develop. Healthy corms planted in infested soil become infected chiefly through the roots, both fibrous and contractile, or more rarely, through the leaf bases.

Numerous isolations and inoculation experiments have proved that a *Fusarium* is the cause of the vascular rot. The observed variations in cultural and morphological characters of the numerous isolates seem insufficient to warrant their separation into varieties or forms, since all have in common the most important character, the ability to cause a specific disease in the gladiolus.

The essential characters of this *Fusarium* place it, according to the classification of Wollenweber and Reinking, in the section Elegans and sub-section Orthoceras. To avoid establishing a new species it is described as a variety of the type species: *Fusarium orthoceras* var. **gladioli** n. var.

The optimum temperature for growth of the *Fusarium* in culture is 23° to 26° C. In soil artificially inoculated with the *Fusarium*, corm infection occurred at all the temperatures tried (15° to 32° C.). The optimum soil temperature for typical and extensive infection of corms is 22° to 25° C.

Not all varieties of gladiolus are susceptible to this disease. On the basis of the writer's tests, 12 varieties are listed as resistant, 30 as moderately susceptible, and 11 as very susceptible. Experimental infection has been

proved in gladiolus only, but a *Fusarium* very similar to the gladiolus pathogen has been isolated from corms of *Montbretia* and *Tigridia*.

The *Fusarium* can be eradicated from soil with chloropicrin but at present this is expensive for large fields. Selection of clean corms, fungicidal dips for planting stock, and planting in uncontaminated soil, are suggested. The most promising means of control is the selection and use of resistant varieties.

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QUANTITATIVE STUDIES WITH CARBORUNDUM AND ITS USE IN LOCAL-LESION TESTS

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INTRODUCTION

The discovery that leaves of various host plants, when rubbed with a solution of tobacco-mosaic virus, develop necrotic lesions at the point of entrance of infective units into the cells has afforded to plant virus research one of its most useful techniques (3). Several improvements on the original technique that were mostly directed towards the elimination of certain experimental errors have been made. Today the technique of estimating virus concentration by local-lesion tests is widely used by plant virologists. It is well known that the study of any virus is greatly facilitated if a test plant giving satisfactory local lesions is available. More than a dozen plant viruses are known to produce local lesions suitable for measuring virus activity (5).

It is generally accepted that, when the leaf surface of a host is rubbed with a virus suspension, the infective units gain entrance into wounded cells of the epidermis and the trichomes. However, only a relatively small number of cells in the surface of the leaf will be wounded in the proper manner to support lesion development. Without considering the susceptibility of the host, the number of lesions obtained in the inoculated leaves is influenced by two main factors: the number of infective units present in the inoculum and the number of cells that are wounded in the right manner. It is clear that the higher the number of infective units present in the inoculum the greater the chance that some will get into properly injured cells.

It has been pointed out (12) that only a small fraction of the infective units of an inoculum succeed in causing lesions. Most of them are lost on the surface of the leaf, in the inoculating pad, etc. It is therefore clear that the number of lesions will increase as the number of appropriate entry points is increased. While the virus concentration may be readily controlled within certain limits, it is not so easy to control the number of entry points. However, it is possible to increase the number of appropriate entry points by the use of abrasives.

The first abrasive used for plant virus inoculation was sand (2, 10). It was found later (8, 9) that carborundum was of great value as an abrasive, and today its use is widespread for the transmission of viruses difficult to pass mechanically. The carborundum or other abrasive particles are believed to make small openings in the epidermal cells or hairs that afford an entrance for the infective units without killing the cells or wounding them severely (9).

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It is surprising that, in spite of a few good results obtained with abrasives in the inoculation of viruses causing local lesions (9, 10), carborundum has been little used in determinations of virus activity. Its use has been restricted almost entirely to the mere transmission of virus diseases. Black (1), working with potato yellow-dwarf virus, seems to have been the first to use carborundum regularly in local-lesion tests.

It is the purpose of this paper to show that, by using carborundum in local-lesion tests, a manifold increase in the number of lesions is obtained on certain host plants with different viruses. The use of carborundum renders the test more sensitive, and this permits estimation of the concentration of virus preparations that could not be measured satisfactorily otherwise.

MATERIALS AND METHODS

Some of the tests herein reported were carried out at the Department of Horticulture, University of Wisconsin. Others were performed at the Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, N. J.

Viruses Used

Three viruses were used in the tests: ordinary tobacco-mosaic virus (*Marmor tabaci* H. var. *vulgare* H.), cucumber-mosaic virus (*M. cucumeris* H. var. *vulgare* H.), and severe-etch virus (*M. erodens* H. var. *severum* H.).

The inocula were obtained by crushing leaves of diseased plants in a mortar and straining through cheesecloth. The plant extract was then centrifuged.

Test Plants

Plants of *Nicotiana glutinosa* L., of the hybrid *N. tabacum* L. × *N. glutinosa* (6), and of *Phaseolus vulgaris* L. var. Early Golden Cluster were used for tests with ordinary tobacco-mosaic virus. *Vigna sinensis* (L.) Endl. var. Black was used exclusively for cucumber-mosaic virus. *Physalis peruviana* L. was used for the inoculations with severe-etch virus (5).

On the tobacco hybrid and *Nicotiana glutinosa*, 5 or 6 consecutive leaves, as uniform as possible, were used per plant, the other leaves and the top being removed before inoculation. *Phaseolus* and *Vigna* plants were inoculated on the first pair of leaves that developed above the cotyledons when they were almost three-fourths of their full size. This generally was 10 days after sowing. Plants of *Physalis peruviana* were used when they had about 6 good-sized leaves.

Inoculation

Inoculations, with the exception of those on *Vigna*, were made by the half-leaf method (10). The carborundum treatment and control were inoculated on the two halves of the same leaf. Inoculations were made in such a way that each treatment or control would occur the same number of times on the right and left halves. The position on the plant also was

TABLE 1.—*Influence of carborundum on the number of local lesions produced on the hybrid, Nicotiana tabacum × N. glutinosa by tobacco-mosaic virus diluted to various concentrations*

Experiment No.	Dilution,[a] treatment, and average number of lesions[b]									
	10^{-4}		10^{-5}		10^{-6}		10^{-7}		10^{-8}	
	Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control
1	288.6	11.6	30.5	0.6	9.3	0.7	.....	.....	.....	.....
2	262.7	5.2	38.2	1.0	20.8	2.6	.....	.....	.....	.....
3	215.7	15.1	18.8	2.1	7.2	0.5	5.2	0.2	.....	.....
4	174.7	19.2	39.4	5.3	13.1	0.0	1.6	0.0	0.8	0.0
5	378.1	43.4	72.4	5.9	17.6	1.1	1.6	0.0	2.5	0.0
Average	264.0	18.9	39.9	3.0	13.6	1.0	2.8	0.1	1.7	0.0

[a] Diluent distilled water.
[b] Average number of lesions per half-leaf on 10 half-leaves.

TABLE 2.—*Influence of carborundum on the number of local lesions produced on Nicotiana glutinosa by tobacco-mosaic virus diluted to various concentrations*

Experiment No.	Dilution,[a] treatment, and average number of lesions									
	10^{-4}		10^{-5}		10^{-6}		10^{-7}		10^{-8}	
	Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control
1[b]	310.8	10.7	82.8	2.5	11.0	0.3	3.2	0.1	1.5	0.1
2[b]	400.0	8.7	113.3	2.7	32.2	0.5	26.1	0.1	10.3	0.0
3[b]	326.3	16.3	136.1	2.6	31.3	0.3	11.0	0.1	1.7	0.0
4[b]	196.6	3.7	17.2	0.3	4.7	0.0	1.4	0.2	0.6	0.1
5[c]	146.4	2.6	30.4	0.6	2.2	0.0	0.2	0.0	0.0	0.0
6[d]	278.0	8.8	71.1	1.1	6.0	0.1	0.7	0.0	0.5	0.2
Average	276.4	8.5	75.2	1.6	14.6	0.2	7.1	0.1	2.4	0.1

[a] Diluent distilled water.
[b] Average number of lesions per half-leaf on 12 half-leaves.
[c] Average number of lesions per half-leaf on 18 half-leaves.
[d] Average number of lesions per half-leaf on 15 half-leaves.

changed so as to allow the various treatments and controls to occur the same number of times at the different leaf positions. With *Vigna sinensis* the carborundum treatment and control were inoculated always on opposite leaves of the same plant.

Two grades of carborundum were used for the majority of tests: a 600-mesh type manufactured by Braun-Knecht-Heimann Company, San Francisco, California, and a 320-mesh type of silicon carbide manufactured by the Carborundum Company, Niagara Falls, N. Y. Carborundum was applied in different ways: by dusting with a small duster, by sprinkling from a vial with a perforated lid or a vial with cheesecloth around the mouth, and by mixing directly in the inoculum. In general, carborundum was applied after the control inoculations had been performed.

The inoculations were made with a small folded piece of cheesecloth dipped into the inoculum and then gently rubbed on the entire surface of the leaf or half-leaf. Usually 6 strokes were used, but this number was increased in the case of the tobacco hybrid to 10 or 12 strokes. Shortly after inoculation the leaves were rinsed with water. Lesions were usually counted 96 to 120 hours after inoculation except in the case of those produced in *Physalis peruviana*. The lesions obtained on this plant were counted 10 days after inoculation.

EXPERIMENTAL

Effect of Carborundum on the Number of Local Lesions Caused by Ordinary Tobacco-mosaic Virus on the Tobacco Hybrid, Nicotiana glutinosa, and Phaseolus vulgaris

Several trials were performed with these test plants using solutions of tobacco-mosaic virus at various concentrations in water or 0.1 M neutral phosphate buffer. In all experiments performed with the tobacco hybrid, 600-mesh carborundum was used, and with *Nicotiana glutinosa* and *Phaseolus* the 320-mesh type. The results of the experiments performed are presented in tables 1 to 3.

Carborundum increased greatly the number of lesions obtained in these 3 test plants, as may readily be seen from the tables. The increase was greater on *Nicotiana glutinosa* and *Phaseolus* than on the tobacco hybrid. This discrepancy may, however, be due to the difference in the 2 types of carborundum or to other differences, resulting from the fact that the experiments were performed at different places.

The increase in number of lesions over the controls, brought about by the use of carborundum, seemed to vary somewhat at different dilution levels. However, the data are not extensive enough to permit a good comparison of the dilution curves with and without carborundum. It can be said that the curves are approximately parallel except at high dilutions. Figure 1 shows the curves obtained with and without carborundum on the 3 host plants.

In some experiments the use of carborundum increased the variation. This was noticeable only when comparisons were made between samples giv-

[b] Average number of lesions per half-leaf on 12 half-leaves.
[c] Average number of lesions per half-leaf on 18 half-leaves.
[d] Average number of lesions per half-leaf on 15 half-leaves.

ing approximately the same number of lesions per half-leaf. Since the variation tended to increase as the numbers of lesions decreased and since carborundum caused an increase in number of lesions, the controls in general

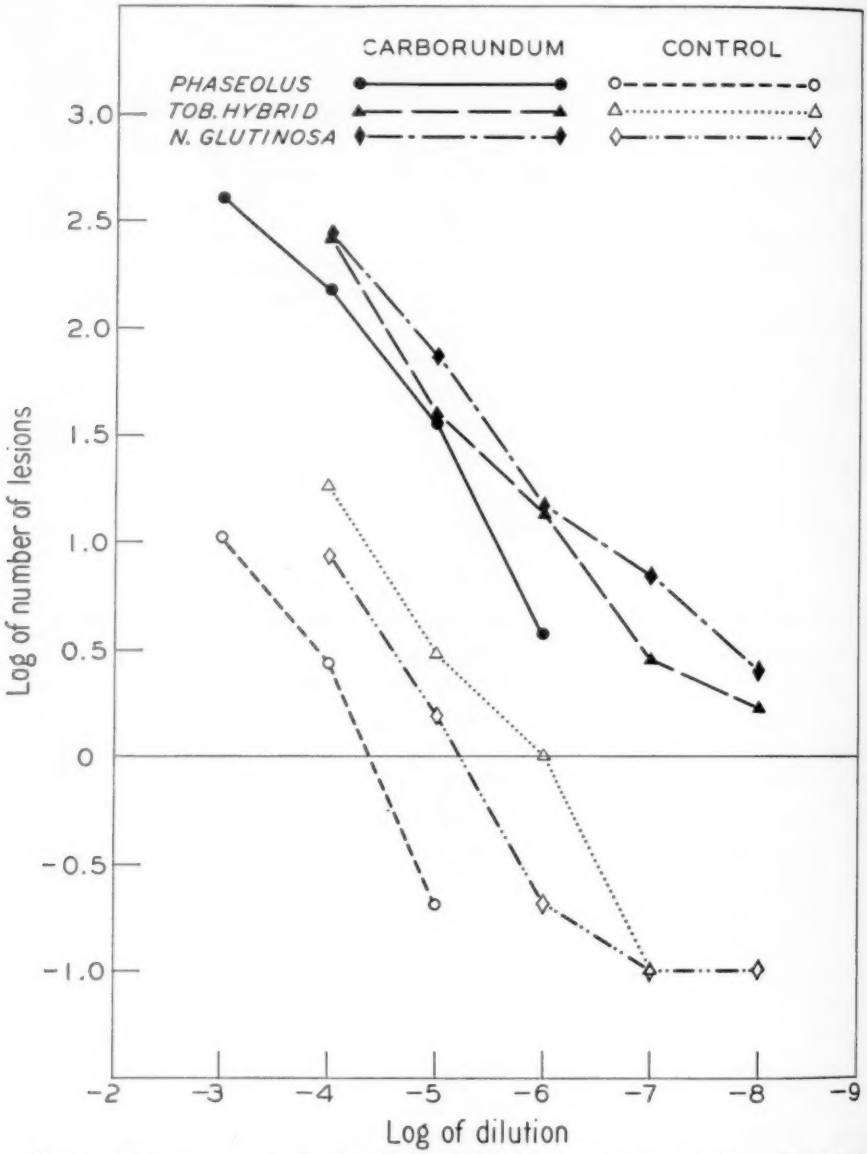

FIG. 1. Dilution curves of tobacco-mosaic virus when inoculated with and without carborundum on *Phaseolus vulgaris* var. Early Golden Cluster, *Nicotiana tabacum* × *N. glutinosa*, and *N. glutinosa*.

showed a greater variation when comparisons were made between samples of the same virus concentration.

With the tobacco-hybrid test plants the increase due to carborundum was more noticeable in the top leaves, the number of lesions being 8.8, 9.2,

TABLE 3.—*Effect of carborundum on the number of local lesions caused by tobacco-mosaic virus on Phaseolus vulgaris var. Early Golden Cluster*

Experiment No.	Dilution,[a] treatment, and average number of lesions[b]							
	10^{-3}		10^{-4}		10^{-5}		10^{-6}	
	Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control
1	460.0[c]	14.4	196.3	4.8	53.6	0.3	6.4	0.0
2	370.0[c]	9.1	124.6	1.2	28.4	0.3	1.7	0.0
3	416.7[c]	7.2	130.6	2.1	24.2	0.1	3.1	0.0
Average	415.6	10.2	150.5	2.7	35.4	0.2	3.7	0.0

[a] Diluent 0.1 M neutral phosphate buffer.
[b] Average number of lesions per half-leaf on 30 half-leaves.
[c] Number of lesions was estimated.

13.6, 19.8, and 33.8 times the number obtained with the control preparations for the 5 leaves, counting from the bottom. On *Nicotiana glutinosa* the increase in number was, at the different leaf positions counting from the bottom, 35.0, 57.4, 59.3, 50.5, 45.0, and 31.1 times those of the control preparations at the same positions.

The Action of Carborundum Is on the Test Plant. Some experiments were made to find out whether carborundum had any action on the virus. A solution of tobacco-mosaic virus at 10^{-4} in distilled water was divided into 3 portions. To 2 portions 600-mesh carborundum was added. After this had been done, the mixture of virus and corborundum was shaken for a while and then the carborundum was filtered out of one of the portions with ordinary filter paper. The 3 preparations—control, virus solution with carborundum, and virus solution to which carborundum had been added and removed—were compared on the leaves of the tobacco hybrid test plant. The results presented in table 4 show that carborundum had no effect on the virus. The preparation to which carborundum had been added and removed gave nearly the same number of lesions as the control.

Methods of Applying Carborundum. A few experiments were carried out to try 3 different methods of applying carborundum: sprinkling or dusting the carborundum on the leaves of the test plant or adding it directly to

TABLE 4.—*Influence of carborundum on the number of local lesions caused by tobacco-mosaic virus on the hybrid, Nicotiana tabacum × N. glutinosa. Inoculum diluted 1: 10,000*

Experiment No.	Treatment and average number of lesions[a]		
	Carborundum added to inoculum	Carborundum added, then filtered out	Control
1	269.9	22.6	25.0
2	210.3	20.8	15.6
3	326.2	33.6	29.1
Average	268.8	25.7	23.2

[a] Average number of lesions per half-leaf on 10 half-leaves.

the inoculum. The results of the trials presented in table 5 show that there were no significant differences among the methods of applying 600-mesh carborundum as tested on the tobacco hybrid test plant.

Comparative Effect of Different Grades of Two Abrasives. These experiments were performed with 5 grades of 2 types of abrasives, carborundum (silicon carbide) and Aloxite (aluminum oxide). Both powders are manufactured by the Carborundum Company.

The inoculum used in these experiments was a solution of tobacco-mosaic virus at $\frac{1}{5} \times 10^{-4}$ in 0.1 M neutral phosphate buffer. The abrasives were added directly to the inoculum and the tests were made on *Nicotiana glutinosa*.

The results presented in table 6 show that a great increase in number of lesions was brought about by all grades of the 2 abrasives. Carborundum of

TABLE 5.—*Influence of carborundum on the number of local lesions caused by tobacco-mosaic virus on the hybrid, Nicotiana tabacum × N. glutinosa, when applied by different methods. Inoculum diluted 1: 10,000*

Experiment No.	Control	Method of applying carborundum and average number of lesions[a]		
		Added to inoculum	Sprinkled on leaves	Dusted on leaves
1	19.3	364.8	267.5	291.2
2	37.1	413.5	484.4	437.1
3	12.4	192.2	266.8	269.5
Average	22.9	323.5	339.6	332.6

[a] Average number of lesions per half-leaf on 30 half-leaves.

500-mesh effected the greatest increase in the 3 trials, being significantly different from the other grades. The other results with carborundum were not significantly different from one another. In the Aloxite series the lowest grade 280-mesh gave the highest number of lesions. The other results do not differ significantly. As a whole, the carborundum grades gave a greater increase than Aloxite. The increase in number of lesions for the 2 abrasives varied from 33 to 122 times the number of lesions obtained for the control.

The Use of Carborundum for Local-Lesion Tests with Cucumber-Mosaic Virus on Vigna sinensis var. Black

Plants of *Vigna sinensis* var. Black have been used for local-lesion tests with cucumber-mosaic virus (7). This test has not, however, been considered very dependable, the results of lesion counts being sometimes so low that they could not be used for estimating virus concentration. The use of carborundum when inoculating cucumber-mosaic virus to *Vigna* gave very good results. Not only was the number of lesions greatly increased, but the counts were uniform and always gave a relatively low standard deviation. Figure 2 gives an idea of the increase in number of lesions brought about by carborundum.

TABLE 6.—*Comparative effect of different grades of two abrasives on the number of local lesions caused by tobacco-mosaic virus on Nicotiana glutinosa. Inoculum 1/5 × 10-4 in 0.1 M phosphate buffer at pH 7*

Experiment No.	Treatment, grade of abrasive, and average number of lesions[a]										
	Control	280		320		400		500		600	
		Carbor.	Aloxite	Carbor.	Aloxite	Carbor.	Aloxite	Carbor.	Aloxite	Carbor.	Aloxite
1	0.3	22.8	.....	25.8	.....	39.6	.....	40.6	.....	37.1	.....
2	1.6	63.3	.....	76.0	.....	72.4	.....	92.6	.....	54.6	.....
3	0.5	73.2	.....	89.6	.....	100.2	.....	159.2	.....	112.4	.....
4	1.1	.....	32.8	.....	19.0	.....	31.4	.....	18.0	.....	20.0
5	0.5	.....	56.9	.....	36.5	.....	49.6	.....	39.5	.....	45.6
6	1.6	.....	90.7	.....	38.1	.....	55.5	.....	23.2	.....	43.2
Average	0.9	53.1	.....	63.8	.....	70.7	.....	97.5	.....	68.0	.....
.....	.....	.....	60.1	.....	31.2	.....	45.5	.....	26.9	.....	36.3

[a] Average number of lesions per half-leaf on 10 half-leaves.

TABLE. 7.—*Influence of carborundum on the number of local lesions caused by cucumber-mosaic virus on Vigna sinensis var. Black*

Diluent	Experiment No.	Dilution, treatment, and average number of lesions[a]							
		Undiluted		1: 5		1: 25		1: 125	
		Carbor.	Control	Carbor.	Control	Carbor.	Control	Carbor.	Control
Distilled water	1	34.2	5.3	14.4	0.4	6.0	0.1	1.4	0.03
	2	75.0	6.6	37.1	0.7	20.6	0.1	4.7	0.0
	3	7.8	0.5	11.3	0.1	2.2	0.0	1.0	0.0
	Average	39.0	4.1	20.9	0.4	9.6	0.07	2.4	0.01
0.1 M neutral phosphate buffer	4	25.1	0.6	53.8	0.5	80.8	0.6	16.6	0.2
	5	64.8	0.9	233.7	5.1	19.5	0.2	1.9	0.0
	6	7.8	0.5	88.3	0.2	21.9	0.1	4.5	0.0
	Average	32.6	0.7	125.3	1.9	40.7	0.3	7.7	0.07

[a] Average number of lesions per leaf on 30 leaves, except for experiments 3 and 6, which are based on 20 leaves.

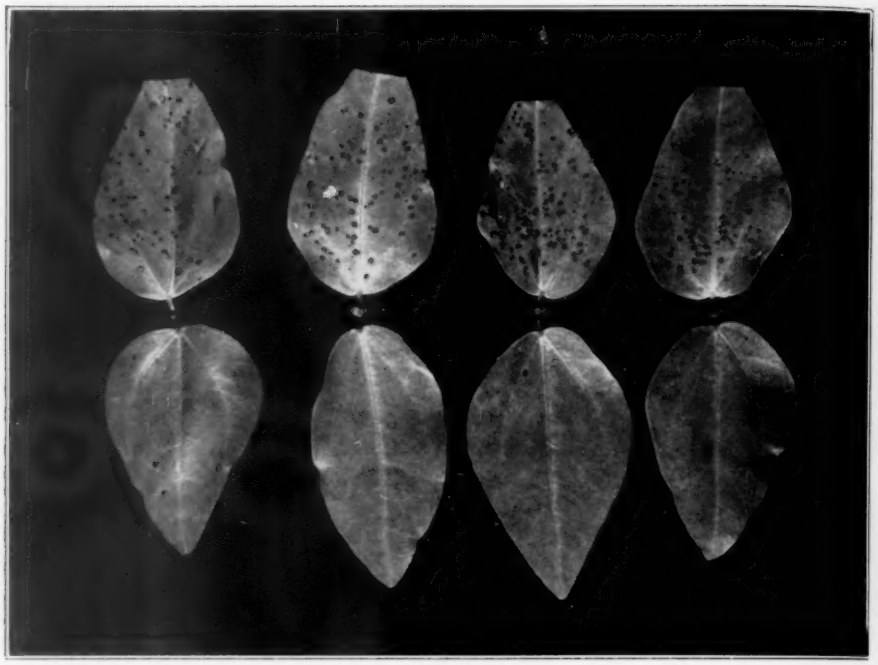

FIG. 2. Increase in the number of lesions obtained by use of carborundum. Plants representative of experiment 6, table 7, dilution 1: 5. (Photograph by J. A. Carlile.)

Two series of experiments were made with *Vigna*. Carborundum 320-mesh was used in both. In the first series distilled water was used as a diluent and in the second the dilutions were made with 0.1 M neutral phosphate buffer. The inoculum was taken from tobacco plants that had shown symptoms for 5 to 15 days.

Examination of the data presented in table 7 shows that the number of lesions obtained for the controls was very low, even when the inoculum was undiluted. In most experiments a satisfactory estimate of the virus concentration could not be based on the figures obtained for the controls. The use of carborundum increased greatly the number of lesions and permitted a comparison between the preparations of different concentrations. Table 8

TABLE 8.—*Standard deviation in percentage of the mean of local lesion tests made with cucumber-mosaic virus and carborundum on Vigna sinensis var. Black*

Experiment No.	Dilution, diluent, and standard deviation in percentage of the mean							
	Distilled water				0.1 M neutral phosphate buffer			
	Undiluted	1: 5	1: 25	1: 125	Undiluted	1: 5	1: 25	1: 125
1	16.1	11.8	15.5	27.9	11.3	7.6	6.4	8.9
2	11.5	11.8	15.5	24.0	10.5	5.6	9.7	15.8
3	16.7	16.8	22.7	30.0	16.7	7.9	10.5	13.3
Average	14.8	13.5	17.9	27.3	12.8	7.0	8.9	12.7

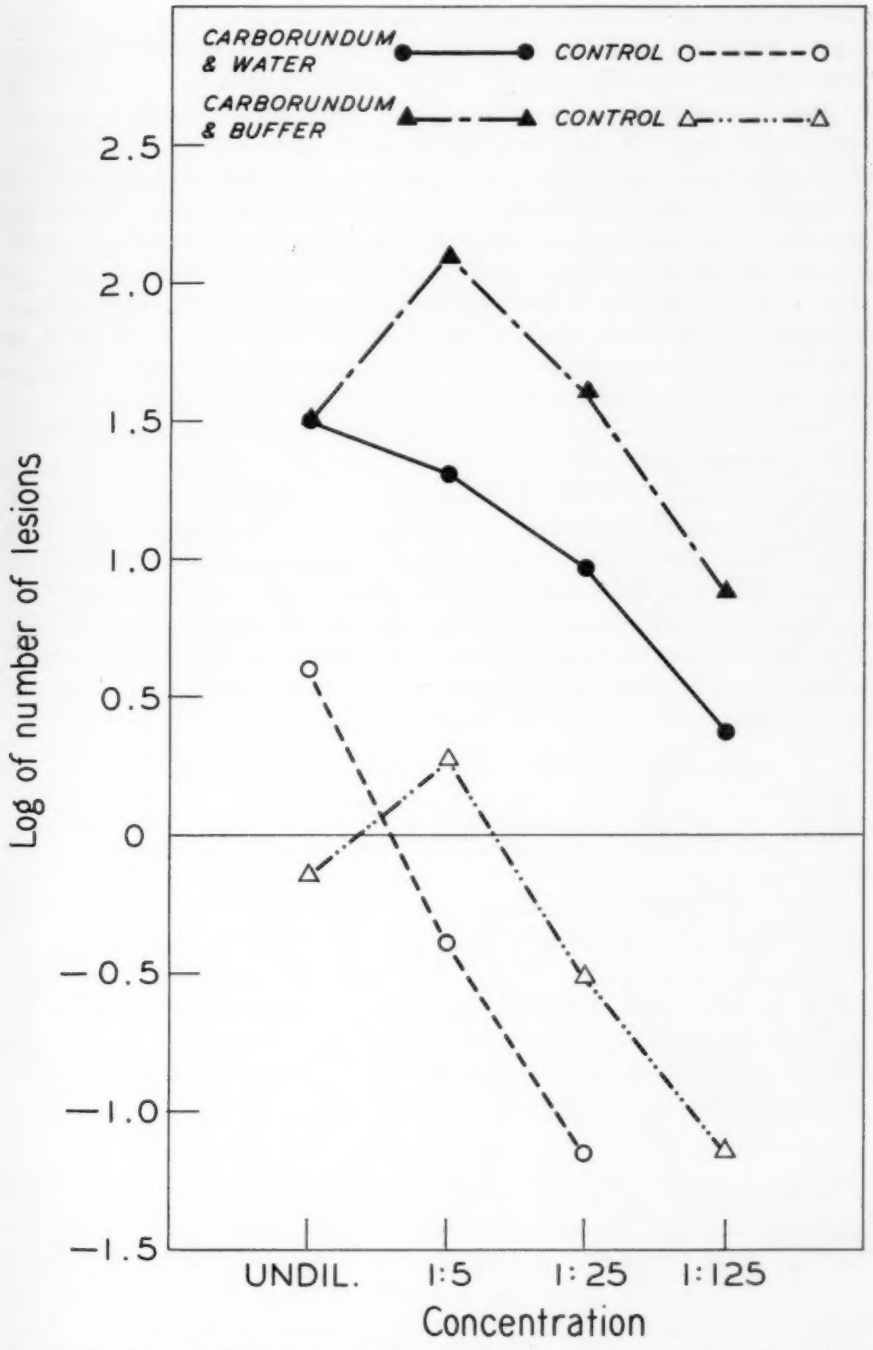

FIG. 3. Dilution curves of cucumber-mosaic virus on *Vigna sinensis* var. Black when inoculated with and without carborundum.

gives the standard deviation in percentage of the mean obtained in these experiments. The deviations are relatively small and permit fairly accurate comparisons.

It is interesting to note that the use of 0.1 M neutral phosphate buffer as a diluent increased the number of lesions in comparison with distilled water. It also decreased the standard deviation even if allowances are made for the greater number of lesions obtained. In the experiments performed with phosphate buffer as a diluent, the samples of virus diluted to 1 : 5 gave a significantly higher number of lesions than the undiluted juice. In one experiment a further dilution to 1 : 25 increased the number of lesions still more. This might suggest that the solutions were not properly buffered until the dilution had been increased to 1 : 25. In one of the experiments using distilled water, the preparation diluted to 1 : 5 also showed an increase over the undiluted inoculum. Figure 3 represents the curves obtained with and without the use of carborundum.

The increase in number of lesions due to the addition of 0.1 M neutral phosphate buffer is apparently to be attributed to the change of pH of the preparation. These results are, however, different from those obtained by Stanley (11). In his experiments, cucumber-mosaic virus was inactivated as the hydrogen-ion concentration was increased or decreased from pH 5.7.

It has been noticed that the use of carborundum in experiments with *Vigna* permits to a certain extent the use of plants of different ages without inconvenience.

Carborundum in Tests with Tobacco Severe-etch Virus on Physalis peruviana

Only a preliminary experiment was made with this virus. The results of the test made during the summer showed that the half-leaves inoculated without the abrasive had an average of 6.5 lesions per leaf, whereas the use of carborundum increased the average number of lesions to 123.8.

According to Holmes (5), it is difficult to obtain a satisfactory number of lesions in tests made during the summer. Under summer conditions the leaves of the plants are thicker and tougher than those grown in other seasons. Carborundum may prove of use in this situation, making the test feasible during the warm season.

DISCUSSION

It had already been shown by Samuel and Bald (10) that 120-mesh sand, used as an abrasive, increased the number of local lesions caused by a yellow-mottling strain of tobacco-mosaic virus on *Nicotiana glauca* Grah. and *N. tabacum* as tested by the iodine technique (4) and also on *N. glutinosa*. Rawlins and Tompkins (9) showed that the number of local lesions caused by tomato spotted-wilt virus on *N. glutinosa* was greatly increased by the use of carborundum. Black (1) reported good results with carborundum when inoculating potato yellow-dwarf virus on *N. rustica*. The experiments

herein reported with 3 viruses and 5 different host plants show that a great increase in the number of local lesions is obtained in these plants by the use of carborundum as an abrasive. Good results with carborundum in the transmission of certain diseases difficult to transmit mechanically have also been obtained by other authors in numerous cases. These results suggest that the entrance of all these viruses into the cells of various host plants when inoculated by rubbing involves the same or a similar mechanism. It seems very likely that carborundum or other abrasives also will increase the number of lesions for other viruses in other host plants.

Carborundum apparently renders the local-lesion tests more sensitive. This may be of little advantage with some viruses. However, under certain circumstances listed below its use may be profitable.

When submitting virus preparations to different treatments in which the titer is so decreased that accurate determinations cannot be made by ordinary means, the use of carborundum might permit such determinations.

Many viruses do not attain a high titer in plant juice. Unless they can be concentrated, their dilution curves cannot be studied satisfactorily by local-lesion tests. Carborundum increases the dilution range and would help in these cases.

The use of carborundum permits tests to be made with more dilute solutions. This at the same time dilutes other substances present in the inoculum and minimizes their possible interference. In testing mixtures of virus-inhibitor or virus-inactivator on dilution, carborundum may be useful.

In tests to determine end points, the use of carborundum might be advantageous.

It also seems advisable to use carborundum when searching for a host plant producing local lesions.

The writer wishes to thank Dr. L. O. Kunkel and Dr. F. O. Holmes for advice and criticism offered during this investigation and in the preparation of the present manuscript.

SUMMARY

Experiments with ordinary tobacco-mosaic virus on *Nicotiana tabacum* × *N. glutinosa*, *N. glutinosa*, and *Phaseolus vulgaris* var. Early Golden Cluster showed that the number of local lesions is greatly increased by the use of carborundum as an abrasive. The same is true for severe-etch virus on *Physalis peruviana* and for cucumber-mosaic virus on *Vigna sinensis* var. Black.

The local-lesion test with cucumber-mosaic virus on *Vigna sinensis* var. Black is greatly improved by use of carborundum, which permits the estimation of virus concentration in samples that could not be measured otherwise. The use of 0.1 M neutral phosphate buffer as a diluent for cucumber-mosaic virus also increased the number of lesions in comparison with distilled water and decreased the standard deviation. Preparations diluted to 1:5

with 0.1 M phosphate buffer gave a higher number of lesions than the undiluted preparations.

Carborundum has no action on tobacco-mosaic virus. Samples to which carborundum was added and removed behaved like the controls.

Three methods of applying carborundum (dusting, sprinkling, and adding to the juice) gave apparently the same results with tobacco-mosaic virus on the hybrid, *Nicotiana tabacum* × *N. glutinosa*.

Five grades of 2 abrasives varying from 280-mesh to 600-mesh were tried. Carborundum 500-mesh gave the best results among the different grades of this abrasive, as did Aloxite 280-mesh in the Aloxite series. However, the results are more or less approximate and any of the grades of either carborundum or Aloxite effected a large increase in number of lesions as compared with the controls on *Nicotiana glutinosa*. As a whole, the carborundum grades gave a higher number of lesions than Aloxite.

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STUDIES OF STEM RUST (PUCCINIA GRAMINIS) FROM POA AMPLA, AVENA FATUA, AND AGROPYRON SPICATUM IN THE PULLMAN, WASHINGTON, REGION[1]

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INTRODUCTION

In the Pacific Northwest during recent seasons stem rust (*Puccinia graminis* Pers.) has been a destructive disease of grasses and cereals. During the past several years reports have been made on the incidence of disease in native and introduced grasses in the Pacific Northwest, and stem rust has always been prominent among the diseases reported (10, 11, 23). In the most extensive of these reports on grass diseases in certain western States (13), 81 species and varieties of grasses were listed as showing more or less infection by stem rust in 1941 alone. It seems probable that more than 100 species of grasses and cereals serve as hosts to *Puccinia graminis* in the Pacific Northwest. It is doubtful if any other region of the United States has stem rust on as wide a range of host species. In spite of this, however, surprisingly little work has been done on host relationships, variety identification, and physiologic race surveys in the stem rust problem in the Pacific Northwest.

In 1940, late-summer and early-fall weather conditions contributed to epiphytotic development of stem rust on many grasses in the Pullman, Washington, area, especially *Agropyron, Elymus,* and *Poa* spp. At this time collections were made of stem rust on wild oats (*Avena fatua* L.), blue bunch wheatgrass (*Agropyron spicatum* (Pursh) Scribn. and Smith), and big bluegrass (*Poa ampla* Merr.) for greenhouse study during the fall, winter, and spring (1940–41). Of especial interest was the stem rust on bluegrasses, which was reported on twelve *Poa* spp. (11). Only one variety of stem rust (*P. graminis poae*) has been known to be commonly associated with *Poa* spp. and this only in Europe, Russia, and a limited region in the central States of this country (28). In 1937 D. C. Smith[3] sent, among other stem-rust collections made at Pullman, a collection on *P. nevadensis* to M. N. Levine,

[1] Investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, in cooperation with the Soil Conservation Service, Division of Nurseries, U. S. Department of Agriculture, and the Washington State Agricultural Experiment Station, Pullman, Washington. Published with the approval of the Director as Scientific Paper No. 555.

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[3] Formerly of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, U. S. D. A.

Pathologist, U. S. Dept. of Agriculture, stationed at University Farm, St. Paul, Minn. Dr. Levine identified the collection as *P. graminis avenae* race 2. This is only the second record of a *Poa* spp. harboring any variety but *P. graminis poae*.[4] It was obvious, then, that the stem rust prevalent on *Poa* spp. and other hosts in the Pullman area merited further study.

MATERIALS AND METHODS

Single-spore cultures of 3 stem-rust collections were obtained by increasing inoculum from a well-isolated sorus of each of these rusts on a sparsely infected, highly susceptible host. The rust from *Poa ampla* was propagated on plants of this species; the rust from *Avena fatua* was propagated on seedlings of Markton oats (C.I. 2053); and that from *Agropyron spicatum* on *Elymus glaucus* Buckl.

The various species and accessions of grasses[5] tested were grown in the greenhouse in 2½-in. pots. Each pot contained 5–15 plants. Inoculations with the 3 cultures were performed in different corners of the same greenhouse, and to some extent in different greenhouses. Whenever the reading for any given accession was similar for more than one of the rust collections, indicating possible contaminations, several accessions of grasses known to be susceptible to only one collection were inoculated with the rust in question. Inoculations were made by spraying fresh spore suspensions with an atomizer or by direct application of spores from heavily infected highly susceptible plants. The inoculated plants were incubated 30–48 hours under several layers of wet sheeting. Infection data were taken 14–16 days after inoculation and classified according to type of infection from 0–4, as described by Stakman and Levine (27).

Most of the grasses employed in these studies are listed in the tables in the following pages. Since it is not practicable to give authorities for binomials listed in the tables and, since immune species are not included therein, a complete list of the species tested follows:[6]

Agropyron caninum (L.) Beauv., *A. ciliare* (Trin.) Franch, *A. cristatum* (L.) Gaertn., *A. dasystachyum* (Hook.) Scrib., *A. elmeri* Scribn., *A. desertorum* (Fisch.) Schult., *A. elongatum* (Host) Beauv., *A. inerme* (Scribn. and Smith) Rydb., *A. intermedium* (Host) Beauv., *A. repens* (L.) Beauv., *A. semicostatum* (Steud.) Nees, *A. sibiricum* (Willd.) Beauv., *A. smithii* Rydb., *A. spicatum* (Pursh) Scribn. and Smith, *A. subsecundum* (Link) Hitchc., *A. trachycaulum* (Link) Malte, *A. trichophorum* (Link) Richt.; *Agrostis alba* L., *A. castellana* Boiss. and Reut., *A. exarata* Trin., *A. scabra* Willd., *A. palustris* Huds., *A. scabriglumis* Boiss. and Reut., *A. spicaventi* L., *A. stolonifera* L., *A. tenuis* Sibth.; *Alopecurus pratensis* L.; *Arrhenatherum elatius* (L.) Presl.; *Bromus arvensis* L., *B. brevis* Nees, *B. brizaeformis* Fisch. and May., *B. carinatus* Hook. and Arn., *B. catharticus*

[4] Stakman (24) successfully transferred stem rust from *Poa nemoralis* to oats, while Stakman *et al.* (29) isolated physiologic races 1 and 2 from *Poa trivialis* collected at Lafayette, Indiana, in 1921.

[5] Mostly contributed by the Pullman nursery unit of the Soil Conservation Service.

[6] Insofar as possible the nomenclature given by Hitchcock (14) is followed.

Vahl., *B. commutatus* Schrad., *B. erectus* Huds., *B. inermis* Leyss., *B. japonicus* Thunb., *B. macrostachys* Desf., *B. macrostachys* var. *lanuginosus* (Poir.) Cos. and Dur., *B. madritensis* L., *B. mollis* L., *B. purgans* L., *B. rigidus* Roth., *B. rubens* L., *B. secalinus* L., *B. squarrosus* L., *B. tectorum* L., *B. tomentellus* Boiss., *B. vulgaris* (Hook.) Schear; *Dactylis glomerata* L.; *Danthonia pilosa* R. Br., *D. semiannulosus* (Labill.) R. Br., *D. unispicata* (Thurb.) Munro; *Deschampsia caespitosa* (L.) Beauv., *D. elongata* (Hook.) Munro; *Elymus canadensis* L., *E. glaucus* Buckl., *E. triticoides* Buckl., *E. villosus* Muhl., *E. virginicus* L., *E. virginicus* var. *intermedius* (Vasey) Bush; *Festuca elatior* L., *F. idahoensis* Elmer, *F. obtusa* Spreng., *F. occidentalis* Hook., *F. octoflora* Walt., *F. ovina* L., *F. rubra* L., *F. scabrella* Torr., *F. Thurberi* Vasey, *F. viridula* Vasey; *Glyceria elata* (Nash) Hitchc., *G. grandis* S. Wats., *G. pauciflora* Presl., *G. striata* (Lam.) Hitchc.; *Holcus lanatus* L.; *Hordeum brevisubulatum* (Trin.) Link, *H. bulbosum* L., *H. gussonianum* Parl., *H. jubatum* L., *H. jubatum* var. *caespitosum* (Scribn.) Hitch., *H. nodosum* L.; *Koeleria cristata* (L.) Pers.; *Lolium perenne* L.; *Phalaris arundinacea* L.; *Phleum phleoides* (L.) Karst., *P. pratense* L.; *Poa ampla* Merr., *P. annua* L., *P. arida* Vasey, *P. attenuata* Trin., *P. bulbosa* L., *P. canbyi* (Scribn.) Piper, *P. compressa* L., *P. curta* Rydb., *P. cusickii* Vasey, *P. epilis* Scribn., *P. glaucifolia* Scribn. and Will., *P. gracillima* Vasey, *P. interior* Rydb., *P. juncifolia* Scribn., *P. nemoralis* L., *P. nervosa* (Hook.) Vasey, *P. nevadensis* Vasey, *P. palustris* L., *P. pratensis* L., *P. scabrella* (Thurb.) Benth., *P. secunda* Presl., *P. sphondylodes* Trin., *P. stenantha* Trin., *P. sterilis* Bieb., *P. sylvestris* A. Gray; *Sitanion hystrix* (Nutt.) J. G. Smith, *S. jubatum* J. G. Smith; *Trisetum flavescens* (L.) Beauv., *T. spicatum* (L.) Richt.

The following cereals were included: *Avena byzantina* C. Koch, *A. fatua* L., *A. nuda* L., *A. sativa* L.; *Secale cereale* L., *Triticum aestivum* L.

The biometrical studies of the urediospores of the 3 stem-rust collections used in the present experiments are based on lengths and widths of 150 spores of each collection. Care was taken to obtain only the superficial spore layers of several mature rust pustules. The spores were mounted in hot lacto-phenol.

RESULTS

One hundred and twenty-three species of grasses and cereals, in 23 genera, were used in the host-range studies of the 3 collections of *Puccinia graminis* from *Agropyron spicatum, Avena fatua,* and *Poa ampla.* A tabular summary of the general reaction of these 23 genera to the 3 stem-rust collections is given in table 1.

A glance at table 1 shows that the strain of *Puccinia graminis* from *Poa ampla* has a surprisingly extensive host range. Within 14 genera, 34 species of grasses and cereals showed more or less susceptibility. Less extensive is the host range of the strain of stem rust from wild oats. Here 18 species in 9 genera proved susceptible. In marked contrast is the very narrow host

range of the stem rust from *Agropyron spicatum* to which 7 species in only 3 genera are susceptible, and these are all of the same tribe (Hordeae).

An analysis of the comparative reaction of the numerous species and

TABLE 1.—*General reactions of 23 genera of grasses and cereals to stem rust collected locally on Poa ampla, Avena fatua, and Agropyron spicatum, near Pullman, Washington*

Reaction of genera	Source of collections					
	Poa ampla		*Avena fatua*		*Agropyron spicatum*	
		No. spp.		No. spp.		No. spp.
Resistant (Infection type 0–2)	*Agropyron*	17	*Agropyron*	17	*Agropyron*	13
	Agrostis	6	*Agrostis*	9	*Agrostis*	9
	Arrhenatherum	1	*Alopecurus*	1	*Alopecurus*	1
	Avena	2	*Bromus*	20	*Arrhenatherum*	1
	Bromus	20	*Danthonia*	3	*Avena*	1
	Danthonia	3	*Elymus*	6	*Bromus*	21
	Elymus	6	*Festuca*	7	*Dactylis*	1
	Festuca	6	*Glyceria*	2	*Danthonia*	3
	Holcus	1	*Holcus*	1	*Deschampsia*	2
	Hordeum	5	*Hordeum*	6	*Elymus*	5
	Lolium	1	*Lolium*	1	*Festuca*	10
	Phleum	1	*Phleum*	2	*Glyceria*	2
	Poa	15	*Poa*	26	*Holcus*	1
	Secale	1	*Secale*	1	*Hordeum*	6
	Sitanion	2	*Sitanion*	2	*Koeleria*	1
	Trisetum	1	*Triticum*	1	*Lolium*	1
	Triticum	1			*Phalaris*	1
					Phleum	2
					Poa	25
					Secale	1
					Trisetum	2
					Triticum	1
Susceptible (Infection type 3–4)	*Agrostis*	3	*Arrhenatherum*	1	*Agropyron*	4
	Alopecurus	1	*Avena*	4	*Elymus*	1
	Avena	2	*Dactylis*	1	*Sitanion*	2
	Bromus	1	*Deschampsia*	2		
	Dactylis	1	*Festuca*	3		
	Deschampsia	2	*Koeleria*	1		
	Festuca	3	*Phalaris*	1		
	Glyceria	3	*Poa*	3		
	Hordeum	1	*Trisetum*	2		
	Koeleria	1				
	Phalaris	1				
	Phleum	1				
	Poa	13				
	Trisetum	1				
		—		—		—
Totals (susceptible)		34		18		7

accessions of grasses and cereals included in this experiment to the three stem-rust collections is given in table 2.

The data in table 2 emphasize those in table 1, showing further the considerable host range differences between the 3 collections of stem rust used in these studies. The range of the Poa rust is especially surprising, since it included grasses in 5 different tribes: *Phalaridae, Avenae, Festucae, Agrostideae,* and *Hordeae.* In contrast, only 1 tribe (*Hordeae*) is repre-

TABLE 2.—*Comparative reaction of various grasses and cereals to cultures of Puccinia graminis isolated from Poa ampla, Avena fatua, and Agropyron spicatum, near Pullman, Washington*

Species and varieties	Accession number	Original hosts		
		Poa ampla	*Avena fatua*	*Agropyron spicatum*
		Infection type		
Agropyron				
dasystachyum	P 7801[a]	0	0	3
elmeri	P 1824	0	2	2
inerme	P 2697	0	1+	0
inerme	P 2792	0	0	4
inerme	P 2522	0	0	2
smithii	F 279	0	0	[illegible]
spicatum	P 3548	0	0	[illegible]
spicatum	P 2719	0	0	4
trachycaulum	F 6	0	0	1
trachycaulum	P 5365	0	0	1
Agrostis				
alba	P 2431	4	0	0
alba	P 2429	4	0	0
alba	P 78	3	0	0
castellana	P 2328	2	0	0
exarata	P 750	3	0	0
palustris	P 4462	2+	2	0
spica-venti	P 75	3	0	0
stolonifera	P 77	2	0	0
striata	P 3176	2+	0	0
tenuis	P 2434	2	0	0
Alopecurus				
pratensis	P 124	4	2	0
Arrhenatherum				
elatius	P 3052	2	4	0
elatius	P 4295	2	4	0
elatius	F 78	0	3	0
elatius	P 1719	0	4	0
Avena				
byzantina				
"Nortex"	C.I. 2382	4	4	–
fatua		4	4	–
nuda				
"Chinese Hulless"		0	4	–
sativa				
"Anthony"	C.I. 2143	0	4	–
Bannock	C.I. 2592	0	4	–
Boone	C.I. 3305	0	1+	–
Fulton	C.I. 3327	0	4+	–
Green Russian	C.I. 2344	0	4	–
Hancock	C.I. 3346	0	1	–
Joanetta	Wn. 2331	0	4	–
Marion	C.I. 3247	0	1	–
Markton	C.I. 2053	0	4	0
Marida	C.I. 2571	0	4	–
Rainbow	C.I. 2345	0	1	–
Red Rustproof	C.I. 458	0	4	–
Iowar	C.I. 847	0	4	–
Victory	C.I. 1197	0	4	–

[a] "P" accessions are those of the Pullman Nursery Unit of the Soil Conservation Service.

"Wn." accessions are those of the Washington Agricultural Experiment Station.

"F" accessions are those of the senior author.

C.I. accessions are those of the Division of Cereal Crops and Diseases, Bureau of Plant Industry.

TABLE 2—(*Continued*)

Species and varieties	Accession number	Original hosts		
		Poa ampla	*Avena fatua*	*Agropyron spicatum*
		Infection type		
Bromus				
brizaeformis	P 2578	2+	1	0
carinatus	P 2725	1	0	0
commutatus	F 193	2+	2	0
macrostachys	F 151	2	0	0
macrostachys var. *lanuginosus*	F 152	2+	2	0
madritensis	F 150	1+	0	0
mollis	F 153	2	2	0
purgans	F 354	1	–	–
rigidus	F 275	1+	1+	0
rubens	F 146	3	1	0
squarrosus	F 143	2	1	0
tectorum	F 165	1+	1+	0
Dactylis				
glomerata	P 187	4	4	0
glomerata	P 952	4	3	0
Deschampsia				
caespitosa	P 779	4	4	0
elongata	P 956	4	4	0
Elymus				
glaucus	F 127	0	0	4
glaucus	P 1848	0	0	4
glaucus	P 1851	0	0	4
glaucus	P 2662	0	–	4
glaucus	P 3572	0	0	4
glaucus	P 3562	1	0	–
sibiricus	P 214	0	0	1
triticoides	P 2714	2	0	0
virginicus	F 133	0	0	1
Festuca				
idahoensis	P 2807	3	0	0
octoflora	P 3130	4	4	0
rubra	P 2347	3	0	0
thurberi	P 5521	2+	3	0
viridula	P 5060	–	3	0
Glyceria				
elata	P 1816	3	–	–
grandis	P 4970	4	0	0
striata	P 3719	3	–	–
Hordeum				
brevisubulatum	P 303	1+	0	0
gussonianum	F 116	2+	1+	2
gussonianum	F 118	2+	0	1
jubatum var. *caespitosum*	F 334	1+	0	1
nodosum	F 122	1+	0	0
nodosum	F 169	2+	0	0
nodosum	P 2723	3	0	0
Koeleria				
cristata	P 1875	4	3	0
cristata	P 2648	4	3+	0
Phalaris				
arundinacea	P 2368	4	3+	0
Phleum				
phleoides	P 2490	3	1	0

TABLE 2—(*Continued*)

Species and varieties	Accession number	Original hosts		
		Poa ampla	*Avena fatua*	*Agropyron spicatum*
		Infection type		
Poa				
ampla	38 PR 395[b]	4	2	0
ampla	38 PR 407	4	1	0
ampla	38 PR 411	4	0	0
ampla	38 PR 419	4	2	0
ampla	38 PR 493	4	0	0
ampla	38 PR 749	4	3	0
ampla	None	4	1	0
ampla	P 846	4	2	0
ampla	P 910	4	1+	0
ampla	P 914	4	1	0
ampla	P 996A	4	2+	0
ampla	P 996B	4	2	0
ampla	P 999	4	2+	0
ampla	P 1000	4	1	0
ampla	P 1880	4	2	0
ampla	P 1883	4	0	0
ampla	P 1888	4	0	0
ampla	P 2534	3	0	0
ampla	P 2716	4	0	0
ampla	P 2800	4	0	0
ampla	P 3317–4	4	0	0
ampla	P 3446	4	0	0
ampla	P 3482	4	0	0
ampla	P 3935	4	0	0
ampla	P 5002	4	0	0
ampla	P 5113	4	1	0
ampla	P 5731	4	0	0
ampla	P 5732	4	0	0
ampla	P 5895	4	0	0
ampla	P 6239	4	2	0
ampla	P 6252	4	0	0
ampla	P 6303	4	1	0
ampla	P 8903	4	2	0
arida	P 5110	3+	0	0
attenuata	P 405	4	0	0
bulbosa	P 2788	4	4	0
bulbosa	P 5011	2	3+	0
bulbosa	P 5727	4	4	0
canbyi	38 PR 399	3	1+	0
canbyi	38 PR 453	3	0	0
canbyi	38 PR 759	4	1	0
canbyi	38 PR 760	2+	0	0
canbyi	38S 1535–1536	3	0	0
canbyi	38S 1539–1540	2+	0	0
canbyi	38S 1541–1542	2+	0	0
canbyi	38S 1543–1544	3	1	0
canbyi	38S 1547–1548	2+	0	–
canbyi	P 844	3+	0	0
canbyi	P 1884	4	2	0
canbyi	P 2756	4	0	0
canbyi	P 3321	4	2+	0
canbyi	P 4052	4	1	0
canbyi	P 6235	4	2	0
canbyi	P 7646	4	0	0
curta	P 2742	4	1	0
cusickii	P 906	2	0	0
cusickii	P 1886	2	0	0

[b] Accessions with designations of this sort are selections made by D. C. Smith, formerly of the Bureau of Plant Industry.

TABLE 2—(*Continued*)

Species and varieties	Accession number	Original hosts		
		Poa ampla	*Avena fatua*	*Agropyron spicatum*
		Infection type		
epilis	P 2743	3	0	0
glaucifolia	P 5506	4	4	0
gracillima	P 850	4	1	0
gracillima	P 1904	3+	1	0
interior	P 5502	1	0	0
juncifolia	38 PR 401	4	2+	0
juncifolia	38 PR 403	4	1	0
juncifolia	38 PR 404	4	0	0
juncifolia	38 PR 405	4	2	0
juncifolia	38 PR 406	4	0	0
juncifolia	38 PR 527	4	1	0
juncifolia	P 837	4	0	0
juncifolia	P 1882	4	2	0
juncifolia	P 1884	4	0	0
nervosa	P 1894	1	1	0
nervosa	P 1898	2	–	0
nevadensis	38 PR 486	4	2+	0
nevadensis	P 5014	4	–	0
nevadensis	P 5017	3+	0	0
nevadensis	P 6281	3	2	0
pratensis	P 426	1	0	0
scabrella	P 2680	3	0	0
scabrella	P 2682	3	2	0
secunda	P 3795	3	0	0
secunda	P 4858	3	2	0
secunda	P 5711	3	1+	0
sphondylodes	P 3021	1	0	0
Secale				
cereale	bulk R. no No.	0	1+	1
"Selection 2B"		0	0	0
"Omskaia"		0	0	0
"Yelislgen"		0	0	0
Sitanion				
hystrix	F 278	0	0	3
jubatum	P 3360	0	0	4
Trisetum				
flavescens	P 461	1	3+	0
spicatum	P 2381	4	4	0
Triticum				
aestivum				
11 varieties[c]		0	0	0
Other species[d]		0	0	0

[c] Athena (C.I. 11693), Federation (C.I. 4734), Hybrid 128 (C.I. 4512), Jenkin (C.I. 5117), Kharkof (C.I. 1442), Oro (C.I. 8220), Requa (C.I. 1554), Rex (C.I. 10065), Tenmarq (C.I. 6939), Turkey (C.I. 6175), Yogo (C.I. 8033).

[d] The species that proved to be immune from all 3 of the stem-rust cultures are as follows: *Agropyron caninum, A. ciliare, A. cristatum, A. desertorum, A. elongatum, A. intermedium, A. repens, A. semicostatum, A. sibiricum, A. subsecundum, A. trichophorum; Agrostis scabra, A. scabriglumis; Bromus arvensis, B. brevis, B. catharticus, B. erectus, B. inermis, B. japonicus, B. secalinus, B. tomentellus, B. vulgaris; Elymus canadensis, E. triticoides, E. villosus, E. virginicus* var. *intermedius; Festuca elatior, F. obtusa, F. occidentalis, F. ovina, F. rubra, F. scabrella; Glyceria pauciflora; Holcus lanatus; Hordeum bulbosum, H. jubatum; Lolium perenne; Phleum pratense; Poa annua, P. compressa, P. nemoralis, P. palustris, P. pratensis, P. stenantha, P. sterilis, P. sylvestris.*

sented in the very limited host range of the stem-rust culture from *Agropyron spicatum*. Some evidence of parallelism may be noticed between the rust from *Poa ampla* and that from *Avena fatua*. However, the two are, nevertheless, sharply distinguished by the reaction of such species as *Agrostis alba, Poa ampla, P. canbyi, P. juncifolia,* and wild and cultivated oats. The Poa rust is innocuous on oats and the oat rust is similarly weak on most of the *Poa* spp.

It is worthy of note that, although nearly 40 accessions of *Poa ampla* were included in these experiments, none showed any resistance. This was disappointing, since the heavy infestations of stem rust in plots and increase fields of this grass had prompted an attempt to find resistant accessions that might be substituted for the highly susceptible accessions now being used. On the basis of the results obtained it seems unlikely that any marked resistance of *P. ampla* to stem rust will be obtained by selection.

Comparative Morphology

Since it has been shown (17) that spore morphology can be used as additional evidence in the identification of varieties of *Puccinia graminis,*

TABLE 3.—*A comparison of lengths and widths of urediospores of the varietal complexes of Puccinia graminis with those of three collections of stem rust from Agropyron spicatum, Avena fatua, and Poa ampla*

Varietal complexes or isolates from local stem rust collections	Mean		Number of measurements
	Length	Width	
Rust varieties reported in literature:			
P. g. tritici (17)[a]	32.40 ± 0.19	19.79 ± 0.06	200
secalis (17)	27.14 ± 0.14	17.19 ± 0.06	200
avenae (17)	28.50 ± 0.15	19.94 ± 0.07	200
phleipratensis (17)	23.95 ± 0.12	16.88 ± 0.06	200
agrostidis (17)	22.37 ± 0.12	15.68 ± 0.05	200
poae (28)	18.64 ± 0.10	15.78 ± 0.07	100
Rust strains isolated from:			
Agropyron spicatum	29.35 ± 0.13	16.98 ± 0.09	150
Avena fatua	25.98 ± 0.09	17.08 ± 0.06	150
Poa ampla	24.81 ± 0.11	16.18 ± 0.06	150

[a] Refers to Literature cited.

biometrical studies were made of the urediospores of the 3 cultures of stem rust. One hundred and fifty mature spores of each collection on a highly susceptible host were measured for length and width. A comparison of the measurements thus obtained with the established measurements (17, 27) of the 6 varieties of *Puccinia graminis* recognized as occurring in the United States, is shown in table 3. As will be mentioned later the collections of stem rust on which the present paper is based do not conform biometrically to any of the 6 varieties of *P. graminis*. However, since Levine (18) and Waterhouse (37) have shown that some physiologic races within the rust variety *tritici* differ significantly in spore size and Bailey (1) and Waterhouse (37) have demonstrated the same thing in the case of *P. graminis*

avenae, these local collections may quite possibly be within the range of variation expected for the various physiologic races of the several varieties of *P. graminis*.

DISCUSSION

In view of the host ranges of the 3 collections of stem rust included in these studies the question of varietal identity becomes of great interest.

The stem rust culture from *Poa ampla* obviously is not *Puccinia graminis poae*, as commonly known. For this variety it has been shown by Eriksson, (4, 5, 6, 7), Eriksson and Henning (9), Jaczewski (15), and Stakman and Levine (28) that only a limited number of *Poa* spp.[7] serve as congenial hosts. Attempts to infect grasses in several other genera have failed. The present work with the single spore culture of stem rust from *P. ampla* shows conclusively that a large number of *Poa* spp. are among its hosts. Several of the species shown by previous investigators to be hosts to *P. graminis poae* were included but all were either immune from or highly resistant to the *P. ampla* rust. It also has been shown in the present investigations that many other species, in 13 other genera, are also more or less susceptible to this polyvorous strain of *P. graminis*. Many of these species and genera have been shown by several investigators[8] to be susceptible to *P. graminis avenae*. The complete susceptibility of Nortex oats (*Avena byzantina*) further suggests the variety *avenae*. However, the demonstrated immunity of 16 other varieties of cultivated oats, including Markton and Victory, strongly indicates a new physiologic race of *P. graminis avenae*.[9] The susceptibility of certain species of *Agrostis, Alopecurus, Dactylis*, and *Koeleria*, listed by Fischer and Levine (12) as hosts to *P. graminis agrostidis* suggests the possibility that the *P. ampla* rust might belong to that variety. On the other hand, the *P. ampla* rust has certain hosts not recognized for *P. graminis agrostidis*, namely, *Avena byzantina, A. fatua, Deschampsia* spp., *Festuca* spp., *Glyceria* spp., *Hordeum nodosum, Phalaris arundinacea, Phleum phleoides, Poa* spp., and *Trisetum spicatum*. The host range also has certain species in common with *P. graminis phlei-pratensis* (31, 33), but the immunity of the 3 accessions of *Phleum pratense* from the stem rust from *Poa ampla* would seem to preclude the possibility of this rust being *P. graminis phlei-pratensis*. By no stretch of the imagination could the *P. ampla* rust be considered as *P. graminis tritici* or *P. graminis secalis*.

A comparison of the urediospore morphology of the *Poa ampla* rust with the established biometric constants (17) of the 6 varieties of *Puccinia graminis* in the United States (Table 3) does not aid in determining the variety of stem rust concerned. The measurements of the rust in question do not conform to those of any of the varieties of *P. graminis*. The nearest

[7] *Poa annua, P. chaixii* Vill., *P. caesia* Smith, *P. compressa, P. fertilis, P. nemoralis, P. palustris, P. pratensis, P. serotina* Ehrh., *P. triflora* Gilib. (The latter two are cited by Hitchcock (14) as synonyms of *P. palustris*.)

[8] See footnote 11.

[9] According to correspondence with M. N. Levine this new race would have the key number 14.

approach is to *P. graminis phlei-pratensis* and *P. graminis agrostidis* but, as already pointed out, these varieties are excluded because of marked host range differences.

It seems, then, that the culture of stem rust from *Poa ampla* might be identified with the variety *Puccinia graminis avenae*. Other varieties of *P. graminis* have been named in Europe and Russia[10] but the validity of at least some of these seems to be in question, and insufficient is known of their host range to permit comparison with the *Poa* rust in question.

A comparison of the present results on stem rust from *Avena fatua* with the work of previous investigators leaves no doubt that it belongs to *Puccinia graminis avenae*. All but 3 of the 17 varieties and species of oats were susceptible. The susceptible reaction of species of *Arrhenatherum, Dactylis, Deschampsia, Festuca, Koeleria, Phalaris, Poa,* and *Trisetum* are largely in accord with the earlier published records for *P. graminis avenae*.[11] What differences do exist can easily be attributed to variations in grass host range of the various physiologic races within *P. graminis avenae*.

TABLE 4.—*The reactions of Puccinia graminis avenae host differentials when inoculated with stem rust collected on Avena fatua at Pullman, Washington*

Variety	C.I. No.	Infection type
White Tartar	551	2
Richland	787	1+
Sevenothree	3251	4

The identity of the physiologic race of *Puccinia graminis avenae* isolated from *Avena fatua* seemed worthy of determination. The 3 stem-rust differential oat varieties used by Levine and Smith (19) were obtained and inoculated with the single spore culture of stem rust from *A. fatua*. The reaction of these varieties is shown in table 4.

The resistant to moderately resistant reaction of Richland and White Tartar and the high susceptibility of Sevenothree point to physiologic race 2, in the light of the analysis and summary of the 10 known races of oats stem rust given by Levine and Smith (19). These authors show a mean reaction of 2++ for White Tartar to race 2, and in the present studies the reaction of White Tartar to the stem rust was 2. However, this does not seem to be significantly at variance with previous work (19), and, with Richland resistant and Sevenothree completely susceptible, there apparently is no other known race to which the collection of stem rust in question could belong.

The very narrow infection range of the culture of stem rust from *Agropyron spicatum* makes it impossible definitely to identify it with any known variety of *Puccinia graminis*. Only 4 species of *Agropyron*, 1 of *Elymus*, and 2 of *Sitanion* proved susceptible. When the original field

[10] *P. g. airae* Erikss. and Henn. (8), *P. g. calamagrostidis* Jacz. (15), *P. g. aperae* Jacz. (15), *P. g. arrhenatheri* Jacz. (15).

[11] See Literature cited: 1, 2, 5, 6, 7, 8, 12, 15, 16, 22, 25, 30, 31, 32, 33, 34, 35.

collection on *Agropyron spicatum* was brought into the greenhouse the inoculum was sown on *Elymus glaucus* and Oro and Federation wheat. A mesothetic reaction resulted on the wheat varieties, and a completely susceptible (infection type 4) reaction on the *Elymus* species. On the basis of these results it was then supposed that some race of *P. graminis tritici* was involved. Plants of the same accession of *Elymus glaucus* were later lightly infected with the stem rust increased on *E. glaucus* and one of the resulting pustules was chosen as the basis for the single-spore culture. However, as already noted, this single-spore culture was unable to infect any of the 12 wheat varieties, and these were twice inoculated in the seedling stage. Furthermore, Oro and Federation, which had reacted mesothetically to the original composite collection from *Agropyron spicatum,* were repeatedly inoculated in the seedling stage with the single-spore culture, but each time no noticeable infection resulted. From these results it seems probable that *P. graminis tritici* was represented in the original composite collection from *Agropyron spicatum* but not in the single-spore culture propagated on *Elymus glaucus,* the identity of which is, therefore, still in question. On the basis of earlier records (12) the only other known variety to which the single-spore culture could belong is *P. graminis secalis.* However, the resistance or immunity of *Agropyron repens* and 3 rye varieties, as well as one lot of bulk unpedigreed rye, while not entirely precluding the possibility of *P. graminis secalis,* certainly is not in support of that variety.

Numerous investigators[12] have cited many species of *Agropyron, Bromus, Elymus, Hordeum,* and to a lesser extent other genera as susceptible to *P. graminis tritici* and *P. graminis secalis.* The very limited grass-host range of the single-spore culture of stem rust from *Agropyron spicatum* thus makes it seem even more improbable that either of these two stem-rust varieties is concerned. Rather, it seems more likely that a new variety of *P. graminis* may have been encountered that is highly specialized to *Elymus glaucus* and a few species of *Agropyron* and *Sitanion.*

The results described in this paper indicate the presence of anomalous strains of stem rust in the Pacific Northwest. The moderate abundance of barberry bushes in this area provides adequate opportunity for new strains to have developed by hybridization. It seems worthwhile and desirable that intensive study be made of stem rust occurring in this region on a very wide range of grasses, and such investigations are already in progress.

SUMMARY

The results are given of inoculations of numerous grasses and cereals with single-spore cultures of stem rust from *Avena fatua, Poa ampla,* and *Agropyron spicatum* collected in the vicinity of Pullman, Washington.

The 3 cultures of stem rust varied widely in their host ranges: to the *Poa* rust culture, 34 species of grasses and cereals showed more or less susceptibility and these represented 14 genera and 5 tribes; to the *Avena fatua*

[12] See Literature cited: 2, 3, 4, 5, 6, 7, 8, 12, 15, 20, 21, 22, 25, 26, 30, 31, 32, 33, 34, 35, 36.

culture, 18 species in 9 genera and 3 tribes were more or less susceptible; to the *Agropyron* culture only 7 species were susceptible, representing 3 genera and only 1 tribe.

The culture of stem rust from *Avena fatua* was identified as physiologic race 2 of the variety *Puccinia graminis avenae.*

The culture from *Poa ampla* seems best identified with the variety *Puccinia graminis avenae,* but the demonstrated immunity of 16 out of 17 varieties of cultivated oats, including Markton and Victory, would seem to indicate a new physiologic race. It represents a virulent and polyvorous strain, but primarily on grasses.

The culture from *Agropyron spicatum* does not seem to belong to any known variety of *Puccinia graminis.* The immunity of 3 accessions of *Agropyron repens* and 3 of rye indicates that *P. graminis secalis* was not represented, and the immunity of 12 wheat varieties indicates that the variety *tritici* was not involved.

Biometrically, the urediospores of the 3 collections did not conform to the established biometric constants of any of the varietal complexes of *Puccinia graminis,* but might, nevertheless, be component part of some of them.

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CHEMICAL AND PHYSICAL CHARACTERISTICS OF MAIZE COBS IN RELATION TO THE GROWTH OF NIGROSPORA ORYZAE[1]

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Poorly matured ears of maize were found more frequently attacked by *Nigrospora oryzae* (B. and Br.) Petch than were well-matured ears. Nigrospora infection in maize ears usually was initiated in the cob, the fungus later invading the kernels. Except for the relationship between cob pH and incidence of disease discovered by Reddy (4) and the observation by Savulescu and Rayss (5) that susceptible ears contained more moisture in the cob under field conditions than resistant ears, apparently no work has been reported on the nature of cobs in relation to incidence of disease.

Arzberger (1) found that *Nigrospora oryzae* grew well on a wide variety of media, including repurified cellulose, and concluded that such adaptability indicated that the fungus was saprophytic in nature. Much better response of the fungus on nitrogenous media than on media containing starch was obtained by Durrell (2). In this connection he observed that the embryo of the maize kernel was invaded before the starchy endosperm.

An understanding of the relationship between the chemical and physical characteristics of the cob and incidence of infection would help to clarify our knowledge of the disease. Studies were arranged, therefore, to show why the fungus attacked poorly matured cobs more frequently than well-matured ones.

DESCRIPTION AND SOURCES OF COBS AND OTHER MAIZE TISSUES EXAMINED IN THIS STUDY

The inbreds, single crosses, and open-pollinated corn from which cobs were harvested for testing throughout this investigation were chosen on the basis of the maturity of the ears. All the corn was grown at Ames, Iowa, in 1938, 1939, and 1940. An Indiana inbred line, 38–11, was used in certain tests because, due to late-date planting, the ears were harvested in a very immature condition. The inbreds designated as 2, 90, 108, 113, and 116 are experimental lines carried by C. S. Reddy of the Iowa Agricultural Experiment Station. Well-matured ears were produced by 90 and 116. Poorly matured ears were produced by 2 and 113. Inbred 113 was very susceptible to Nigrospora infection in the field; 64 of 68 ears examined were infected in 1940. In the case of inbred 108, 2 ears usually were pro-

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duced to a stalk, the primary ear well-matured and the secondary poorly matured. Os426, L289, and Mc401 are Iowa inbred lines, all of which produced poorly matured ears. B2, an Indiana inbred line, and Pr, an inbred developed by E. W. Lindstrom of the Iowa Agricultural Experiment Station produced ears intermediate in maturity. Single crosses carried under my own experimental numbers, 4228, 1531, and 2317, produced well-matured ears. Cobs of small secondary ears, which were poorly matured, also were used in certain tests.

PHYSICAL DIFFERENCES BETWEEN WELL-MATURED AND POORLY MATURED COBS

It is known that well-matured cobs are harder and woodier than poorly matured ones. It was found that the latter contained more water-soluble substances than did the former. For example, meal made from poorly matured cobs of inbred 38–11 contained 13.0 per cent of water-soluble solids, in contrast to 3.8 per cent in meal from well-matured cobs of the single cross 4228.

Poorly matured cobs were found to have a greater water-absorbing capacity than well-matured cobs. Dry cobs, selected on the basis of maturity, were weighed, soaked in water for 12 hours, wiped with a damp towel, and reweighed. In the first experiment the cobs were selected from field corn on appearance alone. The ratio of weight of water absorbed to cob weight ranged from 1.5 to 6.0 with an average of 2.8 for 18 poorly matured cobs, and from 0.9 to 1.9, with an average of 1.5 for 18 well-matured cobs. In a second experiment cobs from selected inbreds were used. Of 17 poorly matured cobs the ratio of weight of water absorbed to cob weight ranged from 1.6 to 2.6 with an average of 2.1, and a range of 1.1 to 1.9 with an average of 1.5 was obtained for 17 well-matured cobs.

RELATION OF pH TO GROWTH OF NIGROSPORA ORYZAE ON THE COB

Reddy (4) found that cobs of inbreds in which Nigrospora dry rot was prevalent had a higher pH than cobs of inbreds with a low incidence of the disease. In connection with studies[3] on the pathogenicity of this fungus, cob pH was found to be high in immature ears of all inbreds, becoming lower during the season in cobs that matured well but remaining high in poorly matured cobs. To study the effect of the pH of the cob on the growth of the fungus, experiments were arranged in which *Nigrospora oryzae* was grown on well-matured and poorly matured cobs at different pH levels.

Two-gram portions of meal from cobs of the well-matured single cross 4228 or of the poorly matured inbred 113 were added to 50-ml. portions of 2 per cent agar. To some of these portions were added small amounts of dextrose or peptone or both, as shown in table 1. The normal pH of the cobs of single cross 4228 was 4.6, and that of inbred 113, 5.2. These pH levels were interchanged by the addition of lactic acid or sodium hydroxide. The

[3] To be published later.

media thus prepared were autoclaved. *Nigrospora oryzae* was transferred to the media, and the cultures incubated at room temperature for 4 days. Data from 4 replications of this experiment are summarized in table 1.

The experiments with cob-meal agar were checked by parallel experiments with unground cob sections. The results were fully in agreement with those obtained with cob meal. Artificial change in pH had no appreciable effect on the growth of *Nigrospora oryzae* on maize cob. These results point to the conclusion that pH is related to cob maturity, but in itself has no great effect on the growth of the fungus.

CHEMICAL ANALYSES OF COBS OF DIFFERENT MATURITIES

Analyses of well-matured and poorly matured cobs were made to determine differences in composition between the two groups. Apparently sound cobs were selected in all but one case. Samples were arranged in pairs so as to compare well-matured with poorly matured cobs. Analyses were made

TABLE 1.—*Growth of Nigrospora oryzae on media containing meal from poorly matured and well-matured maize cobs, at two different pH values, with and without added nutrients*

Source of cob meal	pH	Response of fungus			
		No nutrient added	0.06 g. dextrose in 50 ml.	0.03 g. peptone in 50 ml.	0.06 g. dextrose 0.03 g. peptone in 50 ml.
Single cross 4228	4.6[a]	+[b]	+	+	++
	5.2	+	+	+	++
Inbred 113	5.2	++	++++	+++	+++++
	4.6	++	++++	+++	+++++

[a] The normal pH is recorded first and the adjusted pH second in each case.
[b] Growth of the fungus is rated on an arbitrary scale on which + represents the poorest and +++++ the best growth obtained.

of oven-dried material according to the methods described by Loomis and Shull (3). The results are reported in table 2. The percentages recorded for each constituent of the cobs are the average of duplicate samples from two separate tests.

The first pair of samples consisted of cobs from primary and secondary ears of inbred 108, harvested in 1938. These two samples are designated as 108p and 108s, respectively. Primary ears of this inbred matured well in the field and were not observed infected by *Nigrospora oryzae*. In years favorable to the growth of the inbred a secondary ear was produced on almost every stalk. These secondary ears were poorly matured, and in 1938 approximately 40 per cent of them were infected by *N. oryzae*.

The second pair of samples in the analyses consisted of meal of well-matured primary ears and of poorly developed secondary ears from the same stalks, the weight of the secondary ears in each case being less than 0.3 that of the respective primary ear. These samples are designated by P (primary) and S.3 (secondary), respectively. Such small secondary ears were frequently infected in the field. This material was harvested in 1938.

The third pair of samples consisted of cobs from secondary ears that weighed at least 0.7 as much as the corresponding primary ear, and cobs from the primary ears. These samples are designated by P′ (primary) and S.7 (secondary). Secondary ears of this size were nearly as free of Nigrospora infection as the primary ears. These ears were harvested in 1938.

During a previous study of the disease, C. S. Reddy of the Iowa Agricultural Experiment Station set aside two samples of cob meal, one from cobs of inbreds in which Nigrospora incidence was low, and the other from inbreds in which infection was frequent. These were included in the analyses and designated as OR and OS, respectively.

TABLE 2.—*Chemical analyses of cob meal from well-matured and poorly matured cobs*

Sample	Amount of each constituent calculated as percentage dry weight of sample						
	Reducing sugar	Nonreducing sugar (as glucose)	Total sugar (as glucose)	Nitrogen	Soluble in 80 per cent alcohol	Residue HCl digestion	Lignin
108p	0.15	0.14	0.29	0.030	3.66	41.5	13.7
108s	0.48	0.43	0.91	0.044	5.89	38.3	12.0
P	0.39	0.14	0.53	0.022	4.07	42.5	14.3
S.3	1.06	1.18	2.26	0.037	8.04	39.8	12.9
P′	0.20	0.13	0.33	0.020	3.55	41.4	12.8
S.7	0.27	0.09	0.36	0.027	4.20	41.5	12.5
OR	0.76	0.42	1.18	0.021	5.03	41.7	17.8
OS	1.03	2.69	3.72	0.039	7.95	38.0	15.5
90	0.22	0.09	0.31	0.028	2.83	40.3	12.3
113	tr.	0.0	tr.	0.025	5.64	43.3	10.0
Diseased	0.88	0.27	1.15	0.092	8.65	36.7	11.9
Not diseased	0.73	0.14	0.87	0.036	4.76	42.6	13.1

The fifth pair of samples in the analyses were from cobs of the inbred 90, well-matured, with no Nigrospora infection in 1938, and from cobs of inbred 113, poorly matured, and with approximately 70 per cent of the ears infected with Nigrospora in 1938. The samples are designated as 90 and 113.

In addition to the samples paired on the basis of maturity, an analysis was made of material from infected cobs separated into portions retted by the fungus and portions in which the fungus had not developed extensively. These two samples are designated diseased and not-diseased.

The data summarized in table 2 indicated that the poorly matured cobs contained more available food than the well-matured ones. In general there was more lignin in well-matured cobs than in poorly matured ones, but the difference was not great. The nitrogen percentage appeared to be somewhat higher in poorly matured than in well-matured cobs.

A physical examination of tissues retted by the fungus showed that much of the substance of the retted part was destroyed. Analyses of retted and nonretted portions of infected cob, summarized in table 2, showed that the

percentages of residue from the HCl digestion and the lignin percentages were about equal for each sample. Further study is necessary to understand the effect of the fungus on the insoluble portion of the cobs.

The low sugar content of cob meal from inbred 113 was unlike that of the other susceptible tissues. This inbred died very early in the field, with ears in a poor state of maturity. The cobs used in the test were harvested late in the fall. It appeared likely that the sugar content of these cobs might have been exhausted by bacterial growth or by other agencies. Collections of ears of inbred 113 were made in the fall of 1939, harvesting the first ears soon after the plants had commenced to die. Two subsequent harvests were made at weekly intervals, and a final harvest was made late in the fall. Cobs from these successive harvests were analyzed for sugar (Table 3).

TABLE 3.—*Sugar content of cobs of inbred 113 at successive dates following death of the plant (1939)*

Date	Percentage total sugar (as glucose)
September 11	2.58
September 18	1.38
September 26	0.53
October 20	0.58
	0.00
	tr.
	0.81

Each analysis was made on a composite meal sample prepared from 6 or more cobs, except for the late harvested ears, analysis of which was made of four separate cobs. The sugar content in cobs of inbred 113 was relatively high early in the fall but decreased rapidly following death of the plant.

GROWTH OF NIGROSPORA ORYZAE ON SUBSTANCES SIMILAR TO SEVERAL COB CONSTITUENTS

To obtain a clearer picture of the manner in which *Nigrospora oryzae* assimilates different cob constituents, the fungus was grown on media containing substances similar to certain of those found in maize cobs. These substances are listed in order of the growth of the fungus as measured by visual examination: 2 per cent dextrose, 2 per cent sucrose, 1.2 and 0.6 per cent peptone,[4] 2 per cent hemicellulose,[5] 2 per cent xylan[6] (the above in Czapek's nutrient solution[7] with 2 per cent agar), 0.5 per cent pectin in Czapek's nutrient solution, cellulose in the form of filter paper,[8] and lignin,[9]

[4] Bacto-Peptone, Difco Laboratories.

[5] Kindly donated by Dr. N. H. Gross of the Bacteriology Department, Iowa State College. The hemicellulose was prepared from straw.

[6] Obtained from Pfanstiehl Chemical Company.

[7] The Czapek's nutrient solution consisted of the mineral constituents only.

[8] Whatman's No. 30.

[9] This was prepared from cornstalks and kindly donated by Dr. L. K. Arnold of the Chemical Engineering Department, Iowa State College. It was purified according to Dr. Arnold's suggestion, by five alternate treatments with HCl and NaOH.

the last two wet with Czapek's solution. With none of these was growth nearly so vigorous as on cob-meal agar or on potato-dextrose agar, employed as checks. Growth on hemicellulose and xylan appeared to be nearly as good as that on the dextrose, sucrose, and peptone. On pectin, growth was very slow, and even slower but still appreciable on cellulose and lignin.

In a further test, growth of *Nigrospora oryzae* on filter paper wet with water was compared with that on filter paper wet with Czapek's nutrient solution. The fungus was allowed to grow for a month or more. When the papers were examined the fungus had sporulated sufficiently to exhibit a distinct, gray color on paper wet with Czapek's solution; but, on the filter paper wet with water, sporulation was so sparse as to be visible only with the aid of the microscope.

The possibility that traces of soluble nutrients influenced the growth of *Nigrospora oryzae* on lignin, cellulose, and pectin, even though each of these had been treated to remove soluble nutrient substances, would not be eliminated without further investigation. It was of interest that growth of *N. oryzae* on media containing xylan and hemicellulose compared well with that on media containing dextrose. Of especial interest was the vigorous growth of the fungus on cob-meal agar and on potato-dextrose agar as compared with that on the other media tested. Certain constituents present in cobs and in potatoes apparently were lacking in all the other substances tested.

PRELIMINARY TESTS WITH COB CONSTITUENTS FAVORING GROWTH OF NIGROSPORA ORYZAE

A few preliminary tests were made to obtain information concerning the nature of the substance in maize cobs favoring growth of the fungus. That substances in poorly matured cobs favored growth of the fungus, rather than that substances in well-matured cobs interefered with growth of the fungus was ascertained by adding cob meal prepared from well-matured and poorly matured cobs to plain agar. Growth on agar containing meal of well-matured cobs was much better than on agar without cob meal but not nearly so good as on agar containing meal of poorly matured cobs. Substances favoring the growth of the fungus occurred to some extent in well-matured cobs.

The substances favoring growth of *Nigrospora* in cobs were largely water-soluble. Growth of the fungus was extensive on media prepared from cob extracts, but sparse on media prepared from the leached cob meal.

Growth of the fungus was found to be slightly better on media containing cob meal from cobs retted by *Nigrospora oryzae* than on media containing equal amounts of meal prepared from poorly matured cobs. Apparently the growth of *N. oryzae* on maize tissues did not exhaust substances favorable to its own growth, nor were there formed heat-stable staling products unfavorable to the growth of the fungus.

Autoclaving cob meal (in 2 per cent agar agar) for as long as 3½ hours at 15 lb. pressure did not make meal from well-matured cobs more favorable

for growth of the fungus, nor did such autoclaving render meal from poorly matured cobs less favorable.

An experiment was arranged to determine whether the substances favoring growth were of organic nature. Portions of cob extract charred at red heat were added to Czapek's nutrient solution with 2 per cent dextrose. Growth of *Nigrospora oryzae* on this medium was not better than that on the nutrient solution alone, while growth in the presence of uncharred extract was profuse. This experiment did not completely eliminate the possibility that certain minerals might be the influential factors in rendering poorly matured maize cobs liable to Nigrospora infection, for the chemical nature of the minerals may have been altered in the charring process.

GROWTH OF NIGROSPORA ORYZAE ON WATER EXTRACTS OF MAIZE COBS

In recording the growth of the fungus on cob-meal media an arbitrary scale must be used. The area of mycelial growth proved unsatisfactory as a criterion in that it did not distinguish between sparse and heavy growth of similar extent. The use of liquid cultures from which mycelial mats could be weighed offered a more satisfactory method of comparison; therefore, in further work, liquid cultures were employed. Tests parallel to those described in this section, but in which cobs and cob-meal agar were employed confirmed the results obtained with cob-meal extract. The principal purpose of the study on water extracts of cobs was to compare the effects of extracts of poorly matured and well-matured cobs on the growth of the fungus. In doing this, 20-ml. portions of Czapek's nutrient solution were placed in 500-ml. Erlenmeyer flasks. The hydrogen-ion concentration was adjusted in all cases to approximately pH 5.0 with lactic acid to reduce bacterial contamination. After the addition of cob extract, and in some cases dextrose, and autoclaving uniform pieces of Nigrospora culture on nutrient agar were transferred to the media, and the cultures were incubated at room temperature for 4 days. At the end of this time the mycelial mats were washed on tared percale disks and dried. The heavier mats were gelatinous in consistency, and it was necessary to remove most of the water from these mats by placing the percale disks on a series of blotters before transferring to the 100° C. oven for final drying. After drying, the percale disks with the mycelial mats were weighed.

The cob-meal extracts, obtained by placing the cob meal in boiling distilled water, contained the leachate of 1 g. of meal in each milliliter of extract. The extract of inbred 38–11, on which the fungus grew very well, was used as an arbitrary standard in testing the effect of the cob extracts on the growth of the fungus. In a preliminary test various amounts of extract from cob meal of this inbred were added to 20-ml. portions of Czapek's nutrient solution (2 per cent dextrose). Amounts of extract greater than 0.4-ml. did not produce corresponding increases in growth of the fungus. Thereafter, in making comparisons, 0.4-ml. portions of extract were used.

TABLE 4.—*Mycelial weights of Nigrospora oryzae grown in Czapek's nutrient solution with and without extract of well-matured and poorly matured cobs*

Dextrose added	Weight of mycelium in milligrams (average, 7–9 samples)			
	Check	Single cross 4228 (well-matured)	Inbred 108 (well-matured)	Inbred 38–11 (poorly matured)
None	1.9	5.3	17.4	45.5
2 per cent	3.1	37.0	58.0	130.8

In the first series of tests the fungus was grown on Czapek's nutrient solution, on Czapek's nutrient solution with 2 per cent of dextrose, on nutrient solution with cob extracts, and on nutrient solution with cob extracts and 2 per cent of dextrose. The growth of the fungus in response to cob extract of the poorly matured inbred 38–11 was compared with that in response to extracts of the well-matured inbred 108 and single cross 4228 (Table 4). Without cob extract there was no essential difference in growth with and without dextrose. With the addition of cob extract, growth was much better in the solution containing dextrose than in solutions containing no dextrose. The best growth of the fungus occurred with the addition of extract of poorly matured cobs to medium containing dextrose. Apparently dextrose and substances present in the cob extract influenced the growth of the fungus.

Cob-meal extracts of several poorly matured and well-matured inbreds and single crosses were compared in respect to their ability to support the growth of *Nigrospora oryzae* in liquid culture. In this experiment dextrose was added to the media throughout. The average weights of mycelial mats, with data on the incidence of infection of cobs used as the source of the extracts, are recorded in table 5. In all tests the best growth of the fungus occurred in the presence of extract of poorly matured cobs.

TABLE 5.—*Growth of Nigrospora oryzae on Czapek's nutrient solution containing two per cent dextrose with extracts of cobs of different maturities*

Source of cob extract	Nigrospora infection in the field. No. of ears		Maturity	Weight of mycelium in mg. (ave. 6 samples)
	Examined	Infected		
Ind. inbred 38–11	.....	[a]	Poor	140.4
Inbred OS426	53	21	Poor	76.1
Inbred OS420	46	20	Poor	63.2
Inbred 2	38	14	Poor	71.1
Inbred L289	43	19	Poor	73.2
Inbred B2	60	0	Fair	42.3
Inbred Pr	54	0	Fair	38.7
Inbred 116	102	2	Good	27.3
Single cross 1531	56	0	Good	24.9
Single cross 2317	64	0	Good	22.6
Check	.....	.....	.....	1.8

[a] Inbred Ind. 38–11 was harvested in a very immature condition, while the ears were still green. No infection was present in these ears at time of harvest; infection has not been detected in maize tissue in green condition.

In these two series of tests growth of the fungus in liquid culture was more profuse with that without cob extract, but considerably more profuse with extracts of poorly matured than well-matured cobs.

Studies are being continued on the nature of the substances, more abundant in poorly matured than in well-matured cobs, which favor growth of *Nigrospora oryzae*.

SUMMARY

A study was made of physical and chemical characteristics of maize cobs in relation to prevalence of infection by *Nigrospora oryzae*. Poorly matured cobs were found to be more frequently infected than well-matured cobs.

Poorly matured cobs were less woody than well-matured cobs. The water absorbing capacity of poorly matured cobs was greater than that of well-matured cobs.

The pH of the cob was found to be an indication of cob maturity. Poorly matured cobs have a higher pH than well-matured cobs.

Poorly matured cobs contain more water soluble substances than well-matured cobs. Chemical analyses showed that poorly matured cobs contain more available food, especially sugars, and less lignin, than well-matured cobs. The high sugar content of poorly matured cobs may be dissipated if harvest of the ears is delayed.

Nigrospora oryzae grew well on media containing sugars, peptone, xylan, and hemicellulose, but only slight growth was obtained on pectin, cellulose, and lignin. Growth on cob-meal agar was far more vigorous than on any of the simpler nutrients.

A substance or substances, other than simple nutrients, present in cobs favored growth of *Nigrospora oryzae*. The substance or substances were present in greater abundance in poorly matured than in well-matured cobs. The substance or substances were water soluble and heat stable, and appeared to be organic in nature.

A close relationship exists between susceptibility to Nigrospora infection and growth of the fungus in response to cob extracts.

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TWO NEW VIRUSES AFFECTING TOBACCO AND OTHER PLANTS

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INTRODUCTION

In this paper are given short descriptions of two apparently new or undescribed viruses. They are probably of little economic significance but are of considerable interest because both viruses appeared in an unknown manner in plants growing inside the insect-proof glasshouses at Cambridge.

The first virus to be described was isolated in mid-winter from a plant of *Arabis hirsuta*, which had been growing for a number of years in the glasshouse and had been used for stock cultures of cabbage mosaic (*Brassica virus 1*, Smith). The second virus was isolated from a noninoculated tobacco plant in the summer of 1942, also growing in the glasshouse.

ARABIS-MOSAIC VIRUS

Properties of the Virus

Thermal Inactivation Point. The virus is inactivated by a 10-minute exposure to a temperature of 60° C., but not by a similar exposure at 50° C.

Dilution End-point. In crude sap expressed from infected White Burley tobacco plants, the virus is infective at a dilution of 1 : 100 but not at 1 : 1,000.

Resistance to Aging. The longevity *in vitro* of the virus is between 48 and 72 hours at room temperature.

Transmission. The virus is sap-inoculable, but is not very infectious; it is more difficult to transmit during hot weather when symptoms tend to be masked. All attempts to transmit the virus by means of the aphids *Myzus persicae* and *Aphis fabae* have failed.

Host Range and Symptomatology

Arabis hirsuta. The symptoms on this plant consist mostly of ring and line patterns of dark-green on a lighter background, together with a certain amount of mottling. No necrosis has been observed. Incidentally, it may be mentioned here that great difficulty has been experienced in transmitting the virus to fresh *Arabis* plants.

Nicotiana tabacum var. White Burley. As a rule there are no definite local lesions formed on the inoculated leaf, although slight chlorotic spots have occasionally been observed. Systemic infection takes the form of scattered chlorotic rings, which may be concentric, on the outer leaves. The central shoot then develops a preliminary pallor or chlorosis, followed by a very characteristic splitting of the leaves, and one side or both sides of the lamina may be stripped away. These leaves invariably become necrotic. This peculiar stripping or shredding of the central leaves, together with a

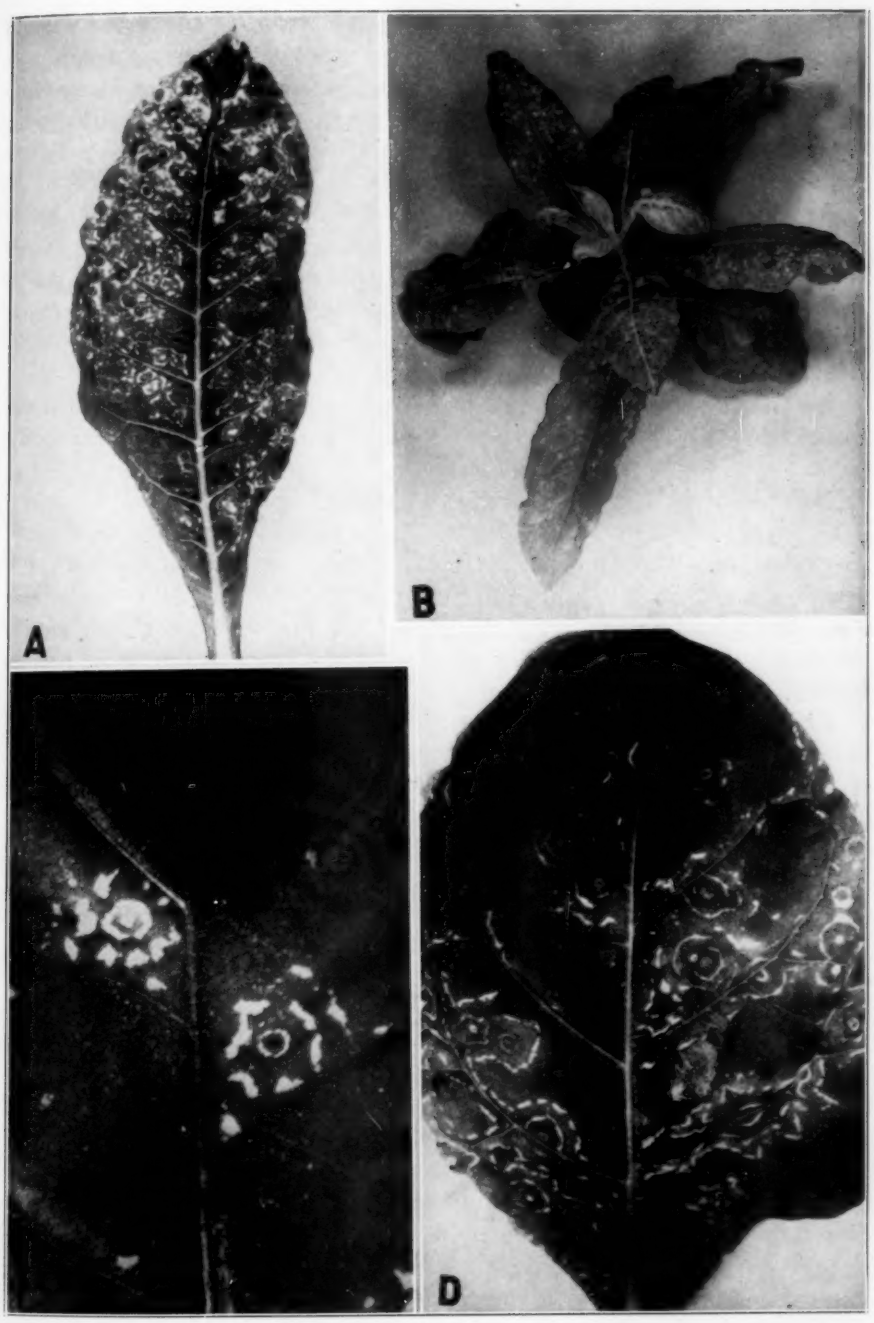

FIG. 1. A. Ring and line patterns produced by the *Arabis* virus on White Burley tobacco. B. Tobacco, White Burley, systemically infected with the *Arabis* virus. Note the characteristic splitting and puckering of the central leaves. C. Incomplete or "broken" rings on White Burley tobacco. D. Systemic symptoms of "broken ring spot" on White Burley tobacco.

reddish necrosis, is the most characteristic symptom of the whole disease (Fig. 1, B). As the central leaves develop, the necrosis increases and the tips pucker up and bend downwards and inwards. At the same time necrotic spots develop on the rest of the plant, and these frequently tend to become ring-like (Fig. 1, A). At this stage the disease is very severe and is particularly so during the winter and at low temperatures.

The incubation period of the virus in the tobacco plant varies from 10 to 21 days, according to the temperature, but may be much longer in very hot weather, when the symptoms are sometimes entirely masked. After a time the plant grows away from the more acute symptoms and looks almost normal, except for a slight mottle and a tendency for the leaves to be dark-green.

Nicotiana glutinosa. On this plant the symptoms are similar to those on the tobacco plant but may be more severe, and the disease is frequently lethal.

Solanum nodiflorum. Chlorotic rings have been observed on the inoculated leaves of this species, but systemic infection does not appear to develop.

Cucumber. The cucumber plant is susceptible to infection with the *Arabis* virus, though the incubation period may be very long, sometimes more than 3 weeks. The symptoms consist of a fairly typical mosaic mottle not unlike that due to cucumber mosaic itself. There are no local lesions formed.

Phaseolus vulgaris, French Bean, var. Canadian Wonder. No local lesions are formed on the inoculated bean leaves, and this is a point of difference from the ring-spot virus, which is the second of the two viruses described in this paper. Systemic infection first develops as small yellow flecks on the young leaves, about a week or 10 days after inoculation. There are, sometimes, raised blisters with the intervening areas pale. The yellow flecks become larger and produce a fairly bright mosaic mottle (Fig. 2, A). This usually is followed by a severe necrosis resulting in the death of the growing points and youngest leaves.

Possible Relationships of the Arabis Virus

Since the virus was first obtained from an *Arabis* plant, which was systematically infected with cabbage mosaic (*Brassica virus 1,* Smith), it seemed possible that the *Brassica* virus might have mutated to give rise to the second virus. Cross-immunity tests, however, do not support this theory. These tests were carried out on the tobacco plant and, since *Brassica virus 1* does not become systemic in tobacco, inoculations were made of *Brassica virus 1* into tobacco plants systemically infected with the *Arabis* virus. The reddish local lesions, characteristic of *Brassica virus 1* on the tobacco plant, duly developed in spite of the presence of the *Arabis* virus.

The fact that the virus will infect the cucumber plant, and also some of its physical properties suggest that it might be a derivative from one or other of the strains of cucumber-mosaic virus propagated at the virus research

station. Again, however, experimental evidence does not bear this out. Tobacco plants systemically infected with the *Arabis* virus were reinoculated with a strain of cucumber-mosaic virus. A strain that gives a bright-yellow mottle was used, so that there could be no doubt whether infection

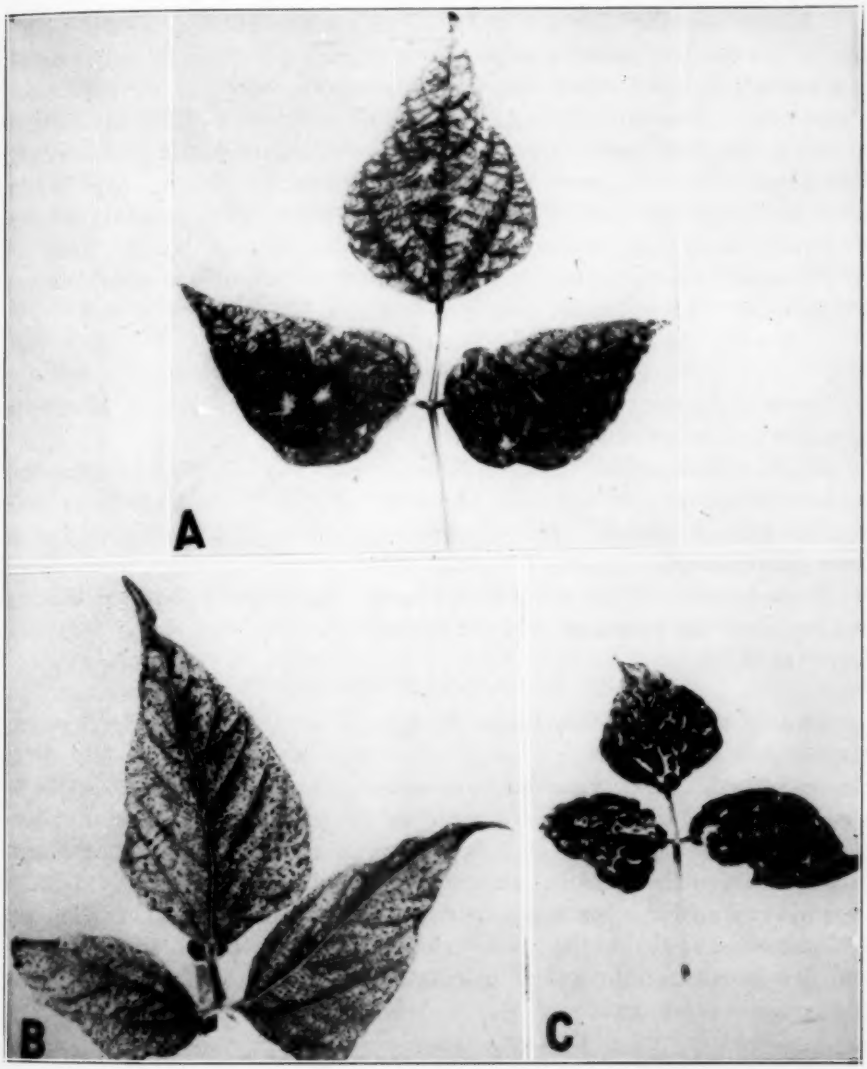

FIG. 2. A. Mosaic mottle produced by the *Arabis* virus on *Phaseolus vulgaris*. B. Numerous small necrotic spots formed on *P. vulgaris* by the broken ring-spot virus. C. Blister mosaic on *P. vulgaris* caused by infection with broken ring-spot virus.

took place. The brilliant mosaic characteristic of this virus developed normally, even though the plants were systemically infected with the *Arabis* virus. Certain other facts militate against the theory that the *Arabis* virus may be a strain of cucumber-mosaic virus. There is the fact that aphids

apparently do not transmit it, and there is its limited host range. For example, it does not infect *Datura stramonium,* a plant that is extremely susceptible to the cucumber-mosaic virus.

Tobacco Broken Ring-spot Virus

A single necrotic ring was observed in a noninoculated tobacco plant, var. White Burley, in the glasshouse. This was cut out and reinoculated to a further series of tobacco plants of the same variety. After a time the plants developed a fairly typical ring-spot disease. It is somewhat difficult to find a new and descriptive name for this virus, since there are already 5 or 6 quite distinct viruses that cause a more or less similar ring spot in tobacco. Recently, Johnson and Fulton[1] have described yet another tobacco ring spot, which they have called "tobacco broad ring spot"; and, since the rings caused by the present virus are frequently incomplete, a suitable name seems to be "tobacco broken ring spot" (Fig. 1, C).

Properties of the Virus

Thermal Inactivation Point. The virus is inactivated by a 10-minute exposure to a temperature of 60° C.

Dilution End-point. Using crude expressed sap, clarified by spinning, positive infections were obtained at a dilution of 1:100 but not at 1:1000.

Resistance to Aging. The virus retains infectivity in extracted sap at room temperatures for about 6 days.

Transmission. The virus is fairly easily transmitted by sap inoculation, but no insect vector so far has been discovered.

Host Range and Symptomatology

Nicotiana tabacum, var. White Burley. Local lesions may develop, but they do not always occur; when they do appear they take the form of a cluster of small single rings on the inoculated leaf. Later, isolated single rings or ring and line patterns develop on the noninoculated leaves. These increase in number and coalesce, so that the leaves eventually become covered with the characteristic pattern (Fig. 1, D). As a rule there is little necrosis or stunting, such as occurs with the tobacco ring spot of Wingard[2] (*Nicotiana virus 12*), in the same variety of tobacco. Local lesions, if present, develop about 5 days after inoculation, followed about a week later by the systemic symptoms.

Nicotiana tabacum, Turkish, var. Kawala. Symptoms in this variety resemble those on White Burley, except that local lesions seem always to form on the inoculated leaves. These lesions usually are single rings, and bear a superficial resemblance to the rings produced by potato virus X on Turkish tobacco.

[1] Johnson, James, and R. W. Fulton. The broad ring-spot virus. Phytopath. 32: 605–612. 1942.

[2] Wingard, S. A. Hosts and symptoms of ring spot, a virus disease of plants. Jour. Agr. Res. [U. S.] 37: 127–153. 1928.

Nicotiana glutinosa. This species appears to be rather resistant to the ring-spot virus; necrotic spots develop, but the disease is not severe and the plant rapidly outgrows the symptoms.

Phaseolus vulgaris, French Bean, var. Canadian Wonder. The reactions of the bean to the ring-spot virus are characteristic and serve to differentiate the ring-spot and *Arabis* viruses, which have some symptoms in common. Very pale chlorotic areas develop on the inoculated leaves within 5 days of inoculation. These are followed a few days later by the systemic symptoms, which take the form of large numbers of minute necrotic spots on the youngest leaves (Fig. 2, B). Frequently, these leaves are killed by the necrosis. Later, a mosaic mottle may develop in which blisters of green tissue stand out from between the necrotic veins, giving the leaves a puckered appearance (Fig. 2, C).

Cucumber. The ring-spot virus produces local lesions on the inoculated leaves of cucumber, and this serves further to distinguish it from the *Arabis* virus. The lesions are white and circular and appear about 5–7 days after inoculation. Systemic symptoms consist of a rather diffuse mottle, less pronounced than that caused by the *Arabis* virus.

SUMMARY AND CONCLUSIONS

Two new viruses affecting tobacco and other plants are described. One was first observed in a plant of *Arabis hirsuta* and the other in tobacco. Both viruses produce diseases of the ring-spot type in tobacco, but the virus from *Arabis* can be distinguished easily by the fact that it induces a characteristic curling and shredding of the central leaves.

The main points of interest concerning these two viruses are their origin and method of spread. Both viruses are new and both appeared in plants that were growing inside the insect-proof glasshouse. Moreover, the disease caused in the *Arabis* plant first appeared in mid-winter. The appearance of these viruses certainly needs some explanation, since they are very uninfectious and no insect vector has been discovered. In consequence, there is no information on their natural mode of spread. Indeed, the viruses would have been lost with the death of the original plants in which each appeared had it not been that they were carefully propagated by mechanical methods of inoculation.

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POLLEN AS A SOURCE OF WALNUT BACTERIAL BLIGHT INFECTION

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(Accepted for publication October 18, 1943)

The severity of walnut bacterial blight (caused by *Phytomonas juglandis*) in California has frequently been ascribed to numerous over-wintering cankers (2, 3, 4). In Oregon, however, the disease has been found to overwinter in diseased buds (leaf and catkin buds) (1). A review of the literature pertaining to the earlier work on walnut blight (3) indicates that this disease was observed on leaf and catkin buds in California without any special significance being attached thereto. In severe outbreaks of the disease, cankers on young shoots were so numerous that they overshadowed the less conspicuous leaf and catkin buds.

Recently, in California, walnut blight was observed in epidemic form, with but few cankers in evidence. The incidence of disease could not be ascribed to such a small amount of inoculum. Examination revealed the presence of the disease (Fig. 1) in dormant catkin and leaf buds and also the presence of blight bacteria on the pollen of diseased catkins.

In a series of experiments, catkins were collected from trees known to have been heavily blighted the previous summer and, after examination under a dissecting microscope, were cultured individually for blight bacteria. Results showed that of the catkins collected on August 20, 1942, 16 per cent yielded live and virulent cultures of *Phytomonas juglandis;* on September 15, 1942, there was 20 per cent; on October 16, 1942, 15 per cent; on November 12, 1942, 30 per cent; on December 23, 1942, 26 per cent; on January 18, 1943, 18 per cent; and on February 20, 1943, 27 per cent.

Of the blighted leaf buds found on the same trees, from 10 to 26 per cent contained living blight bacteria when cultured from August to February at monthly intervals.

Observations on catkins and leaf buds, beginning in March, in the two previously mentioned localities in California, showed an abundance of blighted catkins in all stages of development (Fig. 2). Catkins occupy a strategic position in relation to subsequent infection of foliage and pistillate flowers. Catkins are highly susceptible organs present on the twigs all year, and are subject to infections all year around whenever conditions favor spread of blight. Dormant catkins (or catkin buds) provide a perennial source of water-borne infection, and when mature may shed contaminated pollen, thus providing for aerial dissemination of the blight organism. Healthy catkins may be invaded and killed whenever conditions favor the spread of the disease. Frequently, catkins are partially infected and will shed pollen from the remaining healthy florets. Pollen from the florets on diseased catkins is readily contaminated and can be broadcast alone or in combination with healthy pollen for a considerable distance, causing infec-

tion whenever environmental conditions are favorable for the development of the blight bacterium.

That pollen from blighted catkins may be contaminated by the blight bacteria was shown in a small scale experiment by C. O. Smith (3). How-

FIG. 1. Multiple leaf-bud infections by *Phytomonas juglandis* on Franquette (left) and Payne (right) varieties of walnut. Note the diseased catkin A on the center twig, which was photographed September 3, 1943.

ever, the importance of this aspect of the disease was not stressed, and no experiments were conducted to ascertain its relation to the epidemiology of walnut blight.

FIG. 2. Catkin blight. Note strategic position of the catkins, close to young growth that will bear pistillate flowers.

The writer studied this aspect of the disease during the spring and summer of 1943. Altogether, 150 diseased catkins were cultured directly in the field by shaking the pollen on nutrient agar plates, using three plates for each diseased catkin. Numerous colonies of *Phytomonas juglandis* developed on all plates when incubated at 28° C. The pathogenicity of the cultures was confirmed by inoculation tests on immature nuts in May and June.

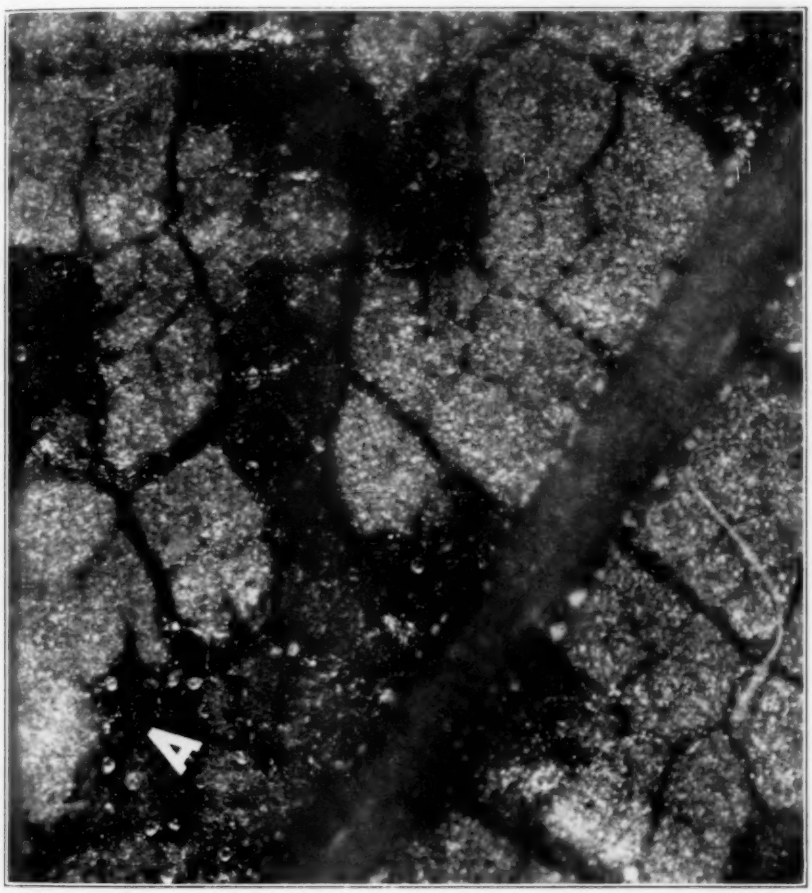

FIG. 3. Walnut blight lesions produced by dusting the leaf with contaminated pollen. Portion of walnut leaf greatly enlarged showing blight lesions produced by dusting with contaminated pollen. Pollen grains shown at A.

Contaminated pollen was mixed with known healthy pollen and this pollen mixture was then tested to determine whether it could induce the disease in pistillate flowers and on walnut leaves. The technique employed by Wood (5) in his walnut pollination tests was adapted for the above-mentioned experiment. The ventral side of leaves was dusted in the leaf experiments. Treated parts were protected with paper bags against contamination. As a check, pollen from healthy catkins was employed in otherwise similar tests. Of 50 pistillate flowers pollinated from healthy catkins,

45 set nuts and no blight infection occurred in these nor in the 5 nuts that dropped. Of 50 pistillate flowers pollinated with contaminated pollen, only 35 set nuts and 20 of these were infected as were also 10 of the 15 nuts that dropped. In all blighted nuts there was only apical type of blight.

In another experiment, leaves were dusted with pollen and bagged. Ten leaves were dusted with pollen from infected catkins and 10 with pollen from healthy catkins. The check leaves were covered with bags, but otherwise not treated. After 1 month no infection had developed in the checks and the leaves dusted with pollen from healthy catkins, while 7 of 10 leaves treated with contaminated pollen bore definite blight lesions (Fig. 3). These lesions yielded cultures of blight organism.

The above-mentioned experiments demonstrate the fact that contaminated pollen may disseminate walnut blight under favorable environmental conditions. Therefore, it is likely that blight infection of nuts may be caused not only by bacteria carried in a film of water during a rainy period from infected leaf buds and catkins, but, also, by contaminated pollen from a partially diseased catkin in which, in the process of natural pollination, is deposited on the stigmas of the pistillate flowers.

The blight organism grows very luxuriantly on agar media containing no other nutrients than walnut pollen. When a culture on this medium is dried completely and held in this condition for several months in the laboratory, it may be revived by plunging a piece of it into a liquid medium.

The blight organism was recovered from dry pollen 4 months after being stored in the vial under laboratory conditions.

The importance of contaminated pollen, together with diseased leaf and catkin buds, may be visualized from Wood's statement (5) that "upon some of the larger Placentia trees as many as 10,000 catkins were counted during a season," and "it was found that single catkins produced from 1,000,000 to 4,000,000 pollen grains."

The prevalence of catkin infection, the presence of viable blight organisms on pollen from infected catkins and the high probability that considerable nut infection may come from this source, emphasizes the importance of infected catkins and contaminated pollen in the overwintering and dissemination of the walnut blight organism.

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A VIRUS DISEASE OF LOVAGE (LIGUSTICUM SCOTICUM)

KENNETH M. SMITH AND ROY MARKHAM

(Accepted for publication June 10, 1943)

INTRODUCTION

During the summer of 1940 the attention of one of us (K. M. S.) was drawn to a plant of lovage (*Ligusticum scoticum* L.) growing in a private garden. The leaves were mottled with patches of light and dark green and showed a typical mosaic disease. Inoculation from this plant to tobacco produced a very severe necrotic disease which invariably killed the latter plant. Since the symptoms produced in tobacco were unfamiliar, an investigation of the virus was undertaken and the results are given here. They show that the lovage mosaic was due to a hitherto undescribed virus.

No attempt has been made to undertake a systematic examination of the complete host range but so far as the inoculation experiments go they suggest that the host range of the virus is not very restricted.

HOST RANGE AND SYMPTOMATOLOGY

Umbelliferae

Ligusticum scoticum L. (*Lovage*). The original infected plant of lovage was a bush of considerable size being several years old. The plant was rather stunted in comparison with healthy plants of the same age and the leaves showed a bold somewhat streaky mottle (Fig. 1, A). There was little necrosis.

We found great difficulty in infecting healthy lovage seedlings with the virus, and only succeeded with 1 plant out of about 100 inoculations. As the attempted infection of lovage seedlings had been made from infected tobacco plants, there was the possibility that the difficulty was due to low virus concentration in the tobacco plants relative to lovage. To test this the following experiment was performed. Twenty tobacco plants, var. White Burley, were inoculated from the original infected lovage plant and 20 similar tobacco plants were inoculated from an infected tobacco plant. In both cases 100 per cent infection was obtained, and there seemed no difference in the infective power of the tobacco and lovage sap.

Daucus carota (*Carrot*) *and Apium graveolens* (*Celery*). Attempts to infect these two plants were unsuccessful.

Solanaceae

Nicotiana tabacum var. White Burley. The tobacco plant is extremely susceptible to infection with the lovage virus, and makes a good indicator or test plant. Local lesions are produced about 7 days after inoculation, but they vary considerably in appearance according to conditions of light and temperature. In one case they take the form of small, dark-red, necrotic

circles with lighter green interiors, and in the other case they appear as large chlorotic circles (Fig. 2, A). Systemic symptoms take about 17 days to develop, and they consist of a very severe necrosis of the veins and midrib (Fig. 2, B), which rapidly spreads and causes the death of the plant. At

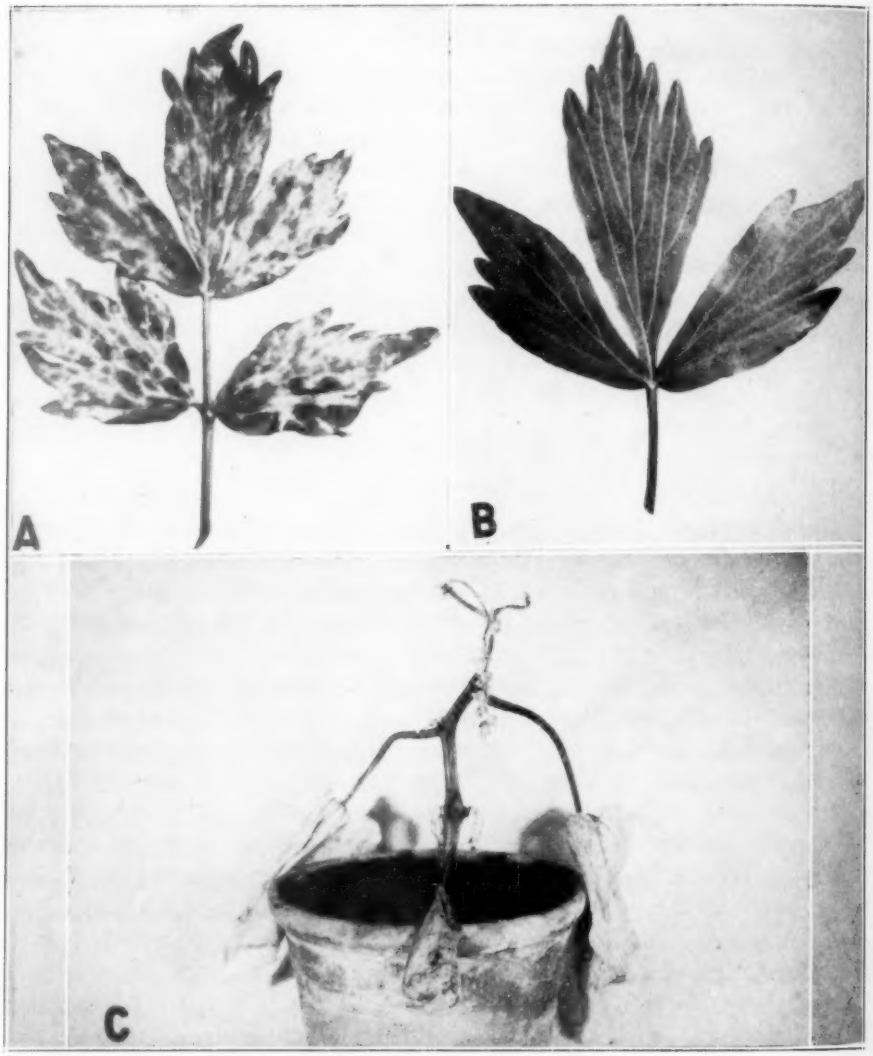

FIG. 1. A. Leaf of lovage plant, showing mosaic mottle. B. Leaf of healthy lovage plant. C. Cucumber plant infected with the lovage virus showing systemic necrosis.

high temperatures the symptom picture may be rather different. Under these conditions there are few local symptoms and the first indication of infection is the appearance of systemic necrosis, which spreads down the petiole of the inoculated leaf and thence reaches the stem. Here it spreads

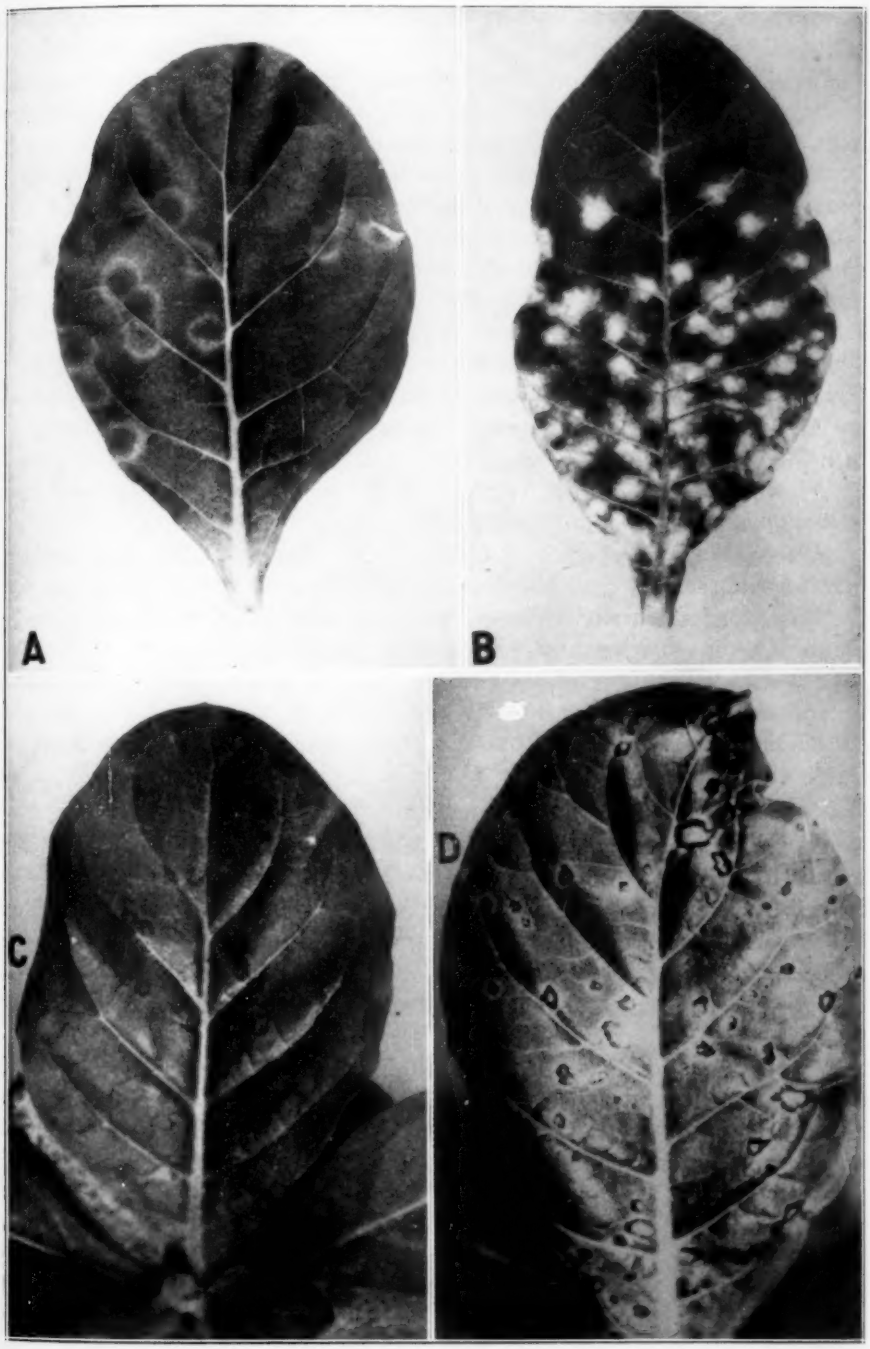

FIG. 2. A. Chlorotic circles on inoculated leaf of White Burley tobacco. B. Systemic infection of White Burley tobacco by the lovage virus. C. Local lesions on *Nicotiana sylvestris*. D. Systemic infection of *N. sylvestris* by the lovage virus.

slowly, both up and down, cutting off the supply of water and food materials so that the plant wilts and dies.

Nicotiana tabacum Turkish, var. Kawala. In this variety of tobacco the leaf symptoms are less pronounced, but, instead, a severe necrosis of the main stem and midribs usually is produced.

Nicotiana glutinosa. Rather faint local lesions are sometimes formed on this species, followed by a faint mottle and some necrosis.

Nicotiana sylvestris. Very distinct and characteristic local lesions develop on this species about 5 days after inoculation (Fig. 2, C). They appear first as shiny glassy spots without a well-defined or necrotic edge. Later, they develop a coppery ring, while still retaining their glassy center. This plant should prove suitable for quantitative studies on the virus. Systemic symptoms develop about a week later and take the form of lesions that are very similar to those formed on the site of inoculation, except that they lack the preliminary glassy stage (Fig. 2, D).

Nicotiana langsdorffii, N. rustica and Capsicum annuum. Local lesions without systemic infection develop on these 3 species.

Lycopersicum esculentum, Tomato, var. Kondine Red. The tomato plant is susceptible to infection, but develops no symptoms and is, in fact, a perfect symptomless carrier of the virus. Infection in other plants is easily obtained by inoculation from such carrier tomatoes.

Datura stramonium. This plant reacts with a faint mottle, there is no necrosis.

Solanum tuberosum. The potato plant appears to be immune from infection.

Leguminosae

Phaseolus vulgaris var. Canadian Wonder. The bean is susceptible to infection with the lovage virus, yellow chlorotic spots developing on the inoculated leaves after 3 or 4 days. Signs of systemic infection develop about a week later as a slight veinal necrosis of the younger leaves, portions of the veins being picked out in yellow. This is followed by a gross systemic necrosis of the youngest leaves, which involves the death of the growing point.

Pisum sativum (Garden Pea). A number of different varieties of garden pea were inoculated, but infection was secured only on the variety English Wonder. In this variety there developed a reddish necrosis of the veins of the youngest leaves and this was followed by the death of the growing point.

Cucurbitaceae

Ridge Cucumber. A slight mosaic mottle develops on the leaves of cucumber plants, and this is followed by a severe necrosis that kills the growing point. The disease produced on cucumber by this virus almost invariably results in the death of the plant (Fig. 1, C).

An experiment was carried out to investigate whether there was any cross immunity between the lovage virus and cucumber-mosaic virus. Six

tobacco plants, systemically infected with a yellow strain of cucumber-mosaic virus, and 6 similarly infected with the type virus were inoculated with the lovage virus. All 12 tobacco plants developed the necrotic symptoms characteristic of infection with the lovage virus. There seems, therefore, to be no relationship between this virus and that of cucumber mosaic.

Malvaceae

Lavatera trimestris (*Garden Mallow*). The cultivated mallow is susceptible and develops a faint yellow mosaic. There is no necrosis.

Cruciferae

Arabis hirsuta (*Rock Cress*). This plant is susceptible, but the symptoms are rather indeterminate; there may be a tendency to ring formation. The virus was recovered from infected *Arabis* plants by inoculation back to other susceptible species.

Some Properties of the Virus

Transmission. With the exception of the lovage plant itself, the virus is easily transmitted by sap inoculation to most of its host plants, but no insect vector is known. Attempts to transmit the virus by means of the aphis, *Myzus persicae*, were unsuccessful.

Thermal Inactivation Point. The virus was inactivated by a 10-minute exposure to a temperature of 60° C., but not to a temperature of 55° C.

Dilution End-point. Using crude extracted sap from infected White Burley tobacco, clarified by spinning, some positive infections were obtained at dilutions of 1 : 100 but not at 1 : 1000. The concentration of the virus in its host plant, therefore, seems to be very low.

Longevity in Vitro. The virus remains infective for about a week in crude expressed sap at room temperature.

DISCUSSION

From a consideration of the symptomatology of the lovage virus, there is little doubt that it has not been previously described. There are one or two points of interest in regard to the virus that might be emphasized here. Its sudden appearance in an isolated plant from which it does not seem to have spread in 3 years, and the fact that it does not appear to be insect-transmitted are rather unusual. It might perhaps be expected that adjacent celery plants, which, after all, are closely related botanically to lovage, would have become infected, but attempts to transmit the virus to celery artificially have proved unsuccessful. Another point of interest is the comparatively high death rate caused by the virus in certain species of plants. Tobacco and cucumber are almost invariably killed, while the growing points in the pea and the bean are destroyed.

The extreme difficulty we have experienced in infecting healthy lovage plants, either from the original lovage plant or from infected tobacco, is also

of interest. In only one instance out of more than 100 attempts did the lovage plant become infected, yet it is quite easy to obtain 100 per cent infection when the virus is transmitted from lovage to tobacco or from tobacco to tobacco. Owing to this apparent difficulty in transmission one cannot state definitely that some plants, such as celery, which have proved resistant to infection, are actually immune from the virus. There is little doubt, had we obtained the virus in the first place in tobacco instead of in lovage, that we should have considered the latter plant immune and should not have persisted to the extent that we actually did in attempts to infect it.

SUMMARY

A new virus affecting lovage (*Ligusticum scoticum* L.) is described. The host range, so far as tested, seems wide and includes plants in the Solanaceae, Umbelliferae, Leguminosae, Cucurbitaceae, Malvaceae and Cruciferae. The virus is sap-transmissible but its natural means of spread are not known. It is inactivated by a 10-minute exposure to a temperature of 60° C. The infectivity of juice from diseased plants is low.

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"FRECKLE," A SPOTTING OF TOMATO FRUITS[1]

H. REX THOMAS[2]

(Accepted for publication November 19, 1943)

A superficial spotting and blemish of ripe tomato fruits, referred to locally as "freckle," commonly occurs on canning tomatoes in Indiana during the latter part of the season. Affected fruits, although off-color in appearance, are seldom graded lower at the cannery, and as a result the disease causes little loss to the grower.

The spots are typically small, $\frac{1}{8}$ inch or less, and have a dark necrotic center surrounded by a yellow border. The side of the fruits exposed directly to the sun is usually more densely spotted. However, the lower side of some fruits and those in the center of densely foliated plants are often affected. The larger spots or blemishes are dark colored and often lack the chlorotic border. They were particularly common on fruits from plants from segregating generations of *Lycopersicon esculentum* Mill. × *L. pimpinellifolium* (Jusl.) Mill. R. E. Lincoln noted apparent differential varietal response on the tomato fruit in the tomato breeding plot at Lafayette, Indiana. Young lesions of anthracnose (*Colletotrichum phomoides* (Sacc.) Chester) (Fig. 1, C) and certain insect punctures, particularly those made by the stilt bug (*Niedes muticus* (Say)), may be confused with freckle.

Two species of *Alternaria* have been most frequently isolated from the affected areas. The predominance of a particular species varied with the time and location where the freckle fruits were collected. In 1939, *Alternaria solani* (Ell. and Mart.) Jones and Grout was frequently isolated from freckle spots on fruits collected at Lafayette and Battle Ground, Indiana (Fig. 1, D). Since that time this species has only occasionally been found associated with freckle. The organism most frequently recovered was one that Charles Drechsler, who examined the cultures, regards as corresponding generally to the species widely referred to as *Alternaria tenuis* Nees. This fungus has not been pathogenic on tomato foliage. It had been repeatedly isolated from aerial spore traps located at Lafayette, Indiana, in 1938 and 1939.

In 1940 and 1941 occasional attempts were made to reproduce freckle symptoms on ripe fruits in the field and on detached green-mature and ripe fruits in moist chambers in the laboratory by spraying the fruit with a water suspension of mycelium and conidia of the above species of *Alternaria*, but

[1] Cooperative investigations of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Department of Botany and Plant Pathology, Purdue Agricultural Experiment Station. Journal Paper No. 122, Purdue University Agricultural Experiment Station.

[2] Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture.

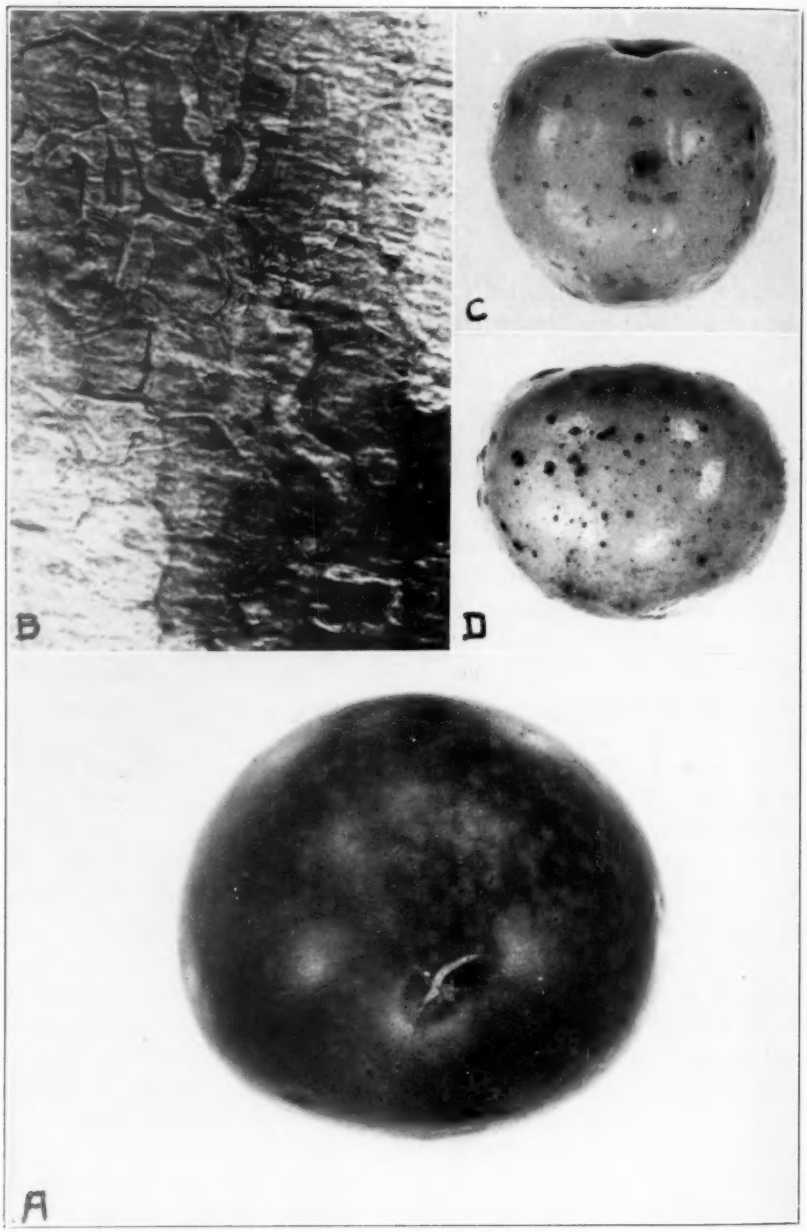

FIG. 1. A. Tomato fruit showing "freckle" symptoms over entire surface. Note chlorotic borders and necrotic centers of spots. B. Fresh mount of epidermal strip from a freckle-affected fruit showing mycelium under the cuticular layer. ×500. C. Tomato fruit showing young anthracnose lesions. D. Young fruit infected by *Alternaria solani* and showing symptoms of a type common in Indiana late in the season.

these attempts were generally unsuccessful. Only a few spots developed and these were all on the green-mature fruit inoculated with *Alternaria solani*. Fruits selected in the field to serve as uninoculated checks frequently developed symptoms during the incubation periods.

In 1942, green, green-mature, pink, and ripe greenhouse fruits were atomized with water suspensions of *Alternaria solani*, *A. tenuis*, a *Penicillium* species, and an unidentified contaminant found in a laboratory culture. The fruits were kept moist for 3 days after inoculation. When *A. tenuis* was used for inoculum, minute spots were visible 4 days after inoculation on the green-mature fruits (then in the pink stage). When the green fruits inoculated with *A. tenuis* began to turn red, spots typical of freckle appeared. Only a few spots developed on the fruits that were ripe at the time of inoculation. The spots on the green fruits resulting from inoculation with *A. solani* were small, raised, and jet black. This type of spotting is typical of infection produced by this fungus on the green fruit in the field, late in the tomato season, and has been described by Gardner.[3,4] Probably only those infections of *A. solani* occurring on the green-mature and ripe fruit are typical of what is referred to as freckle. No spotting was produced by the *Penicillium* species or the unidentified organism.

To study the host-parasite relationships, epidermal strips were removed from freckle-affected fruits obtained in the field. The strips were stained and mounted in a lactophenol solution of acid fuchsin. Mycelium was easily observed in many of the freckle spots (Fig. 1, B). It was in clear focus a short distance beneath the cuticular layer. When paraffin sections were made from epidermal strips and stained with safranin and fast-green, the fungus was observed in a few sections to penetrate through the unbroken cuticle. Many affected areas were located beneath cracks in the skin surface, especially at the bases of broken hairs.

In the affected areas the cells were brown colored and contained brown masses which stained deeply with safranin. What appeared to be oil globules and crystals were observed in the cells. The discoloration seldom extended through more than 3 or 4 layers of cells below the epidermis. Most of the discolored cells, except those in the epidermal layer, were collapsed. The mycelium was observed most commonly in the epidermal layer, but was found also in the cells below. Cell distortion and discoloration apparently occurs in advance of the fungus.

The few attempts to increase the number of infections on the side of the fruit exposed to ultra-violet and infra-red rays were unsuccessful. It is suggested that the predominance of infection on the portion of the fruit exposed to the sun may result from a heavier deposit of air-borne inoculum rather than from any effect from the sun's rays. The greater abundance of freckle late in the season may be explained by the heavier spore load

[3] Gardner, Max W. Indiana plant diseases, 1921. Proc. Ind. Acad. Sci. 33: (1923) 1924.

[4] Gardner, Max W. Indiana plant diseases, 1925. Proc. Ind. Acad. Sci. 36: (1926) 1927.

known to occur in the air at that time, together with the increased exposure of the fruits.

Other fungi may enter the tomato skin and produce a reaction similar to that reported above. Ainsworth *et al.*[5] have described a spotting of tomato fruits caused by a *Botrytis* species under moist conditions. In a study of the structure of tomato skins Groth[6] found patches of brown cells wherever the cuticle or cuticular thickening was cracked, and often the germinating spores of mold fungi were observed in them. The freckle spotting observed in Indiana, however, does not resemble that caused by the organism just mentioned and appears to be due to infection either by *Alternaria solani* or by *A. tenuis*.

[5] Ainsworth, G. C., Enid Oyler, and W. H. Read. Observations on the spotting of tomato fruits by *Botrytis cinerea*. Pers. Ann. Appl. Biol. 25: 308–321. 1937.

[6] Groth, B. H. Alfred. Structure of tomato skins. N. J. Agr. Exp. Stat. Bull. 228. 1910.

MATURE PEACH FRUITS AFFECTED BY LEAF CURL

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(Accepted for publication November 17, 1943)

It appears that *Taphrina deformans* frequently affects the fruits of peach in New York and elsewhere in the United States but is not often observed or recorded on the mature fruits, judging by the paucity of information in the literature. Most records found are of lesions on young or half-mature fruits. In years past the disease has been observed on mature peach fruits at harvest time in New York State by my colleagues Whetzel, Barrus, and Reddick, but not reported. One excellent specimen, preserved in the plant pathology herbarium, was collected in September, 1913. The surface of one fruit was completely involved. Cunningham[1] reported this fungus on mature fruits in New Zealand, adding that this may occur more commonly than is realized.

The first record of lesions on mature peach fruits in the United States appears to be that of Poole[2] who noted affected fruits in the sand hill regions of North Carolina in 1932. He records that when the largest fruits were one-half to three-fourths of an inch in diameter, small hypertrophied leaf-curl lesions were observed confined to the fruit of the varieties Elberta and J. H. Hale, usually on one side and near the calyx end. The disease lesions enlarged throughout the summer, reaching half an inch in diameter. No infections were observed on the early varieties, even when growing in proximity to affected Elberta and J. H. Hale. It is not clear where the inoculum originated nor why the foliage escaped infection. He described the lesions as reddish to purplish in color. Because of no further growth in the diseased areas the fruits were decidedly malformed later in the season because starting in as hypertrophic areas the affected tissues became decidedly atrophic and the surface smooth. Poole[3] mentioned that similar fruit symptoms appeared in Georgia in 1928 and in South Carolina in 1931. The greatest amount of infection encountered was 15 per cent; but, ordinarily, it was less than 2 per cent. Rose, *et al.*,[4] show color reproductions of symptoms on mature fruits. Their suggestion that such symptoms are the result of fungus attacks on maturing fruits seems doubtful.

The discovery that peach-leaf-curl symptoms were exhibited by maturing Elberta fruits in New York in 1943 was made more or less by accident. Due to the previous excessively cold winter very few peaches were produced out-

[1] Cunningham, G. H. Leaf curl, bladder-plum, and cherry-curl. Their appearance, cause, and control. New Zealand Jour. Agr. 26: 85–97. 1923.

[2] Poole, R. F. Late infection of peach leaf curl in the Carolinas. Pl. Dis. Rptr. 16: 171–172. 1932.

[3] See footnote 2.

[4] Rose, D. H., D. F. Fisher, C. Brooks, and C. O. Bratley. Market diseases of fruits and vegetables: peaches, plums, and cherries and other stone fruits. U. S. Dept. Agr. Misc. Pub. 228, 26 pp. 1937.

side of a small area of Niagara County. The peach crop in this area was kept under close observation by the writer during the summer. Although all growers normally spray for leaf-curl control, one grower's Elberta orchard suffered from this disease, which was traceable to the deteriorated sulphur fungicide he used. When examining this orchard early in September it was noted that occasional leaves with curl symptoms minus discoloration still persisted. On closer examination some of the fruits were found to have large reddish lesions covering from one-eighth to three-fourths of the surface area, whereas Poole[3] states that the lesions reached only one-half inch in diameter. The lesions illustrated in figure 1 were relatively shallow, being limited to the outer layers of cells. Many of the lesions developed cracks running usually in a longitudinal or a diagonal direction. The lesions were variable in position, involving areas near the ends of the fruits, strips from end to end, one entire side or one-half the fruit, all one side and

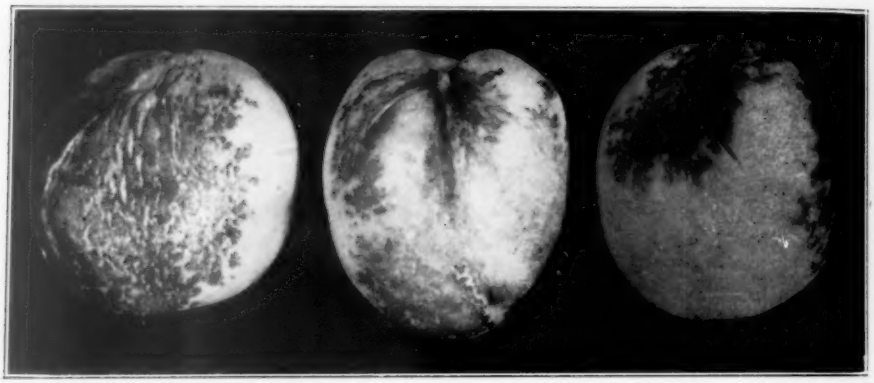

FIG. 1. Leaf-curl symptoms on maturing Elberta peach fruits characterized by striking reddish lesions accompanied by atrophy. Reduced.

portions of the other side, etc. The percentage of fruit affected from tree to tree was ordinarily less than 5 per cent. However, due to prices of 6 dollars or over per bushel, an economic loss resulted in this orchard.

Attempts to demonstrate the leaf-curl fungus in the lesions were all unsuccessful. However, Cunningham,[5] in discussing leaf curl on nectarine fruits in New Zealand states, "Fructifications may develop on these areas, appearing as a delicate bloom; they are unusual, however."

A histological study employing free-hand sections revealed a large reduction in the hairs normally on the surface, which no doubt accounts for the shiny appearance of the affected areas. Cunningham[5] refers to the fact that the lesions ". . . on peaches often appear as if polished, owing to the absence of those hairs which normally cover the surfaces." The reddish, netted discoloration of the lesions on fruits nearing maturity was found to be limited to a shallow layer of cells at the surface, ranging to a depth of about 8 cells. It was further noted that the cells in the region of the lesion

[5] See footnote 1.

were much smaller than normal, possessing thicker walls and more evidence of opaque material within them. Stomates were abundant on both healthy and diseased areas.

In conclusion, the strikingly colorful nature of the lesions observed on mature fruits in New York agree in general with the descriptions of those observed elsewhere. Judging by the nature of its host-parasite relationship, failure to isolate the leaf-curl fungus from old fruit lesions or to observe mycelial elements in connection with them was to be expected. It is not yet clear, however, what the conditions are surrounding the origin and developmental history of lesions on mature peach fruits.

RELATION OF RUST DAMAGE IN SEED FLAX TO SEED SIZE, OIL CONTENT, AND IODINE VALUE OF OIL[1]

H. H. Flor[2]

(Accepted for publication October 22, 1943)

It is estimated that flax rust, *Melampsora lini* (Per.) Lév. reduced the yield of flaxseed in North Dakota in 1942 approximately 25 per cent, or about 2,000,000 bushels. Bison flax, the principal variety grown, is susceptible to North American races of the flax-rust fungus. This variety came into general cultivation in the early 1930's, a period characterized by hot dry seasons, unfavorable for the development of flax rust. Since 1939 rust has become increasingly destructive, and Bison is being replaced by rust-resistant varieties.

Flax rust was generally distributed over North Dakota in 1942, but varied greatly in intensity in different sections of the State and even in different parts of a field. Infection was general in some fields, but in others it was highly variable. Such a variation in intensity occurred in a series of seed-treatment plots at Fargo and offered an opportunity to determine the effect of variations in rust damage on the quantity and quality of oil obtained from the seed.

Bison seed of high quality was used in this test, and the seed treatments with different fungicides had no significant effect on stands. The number of plants ranged from an average of 44 to 55 per foot of drill row. Data secured by Dillman and Brinsmade[3] suggest that in nursery rows, where weeds are not a factor, basal branching compensates for wide variations in stand. In the present tests, actual yields obtained from the various plots corresponded so closely to estimates of the relative injury due to rust, that yields have been used as the criterion of rust damage. Rust-resistant varieties in the nursery yielded about 25 bushels per acre. Rust was estimated to have reduced the yield of the least injured plot of Bison approximately 30 per cent. The yield of the 20 plots ranged from 3.6 to 16.8 bushels per acre. The seed from the different plots ranged in weight from 5.1 to 6.2 grams per 1,000 seeds; in oil content from 36.9 to 39.5 per cent; and in iodine number from 178 to 183.[4] Rust infection was more or less uniform throughout the entire row in the least and in the most severely injured plots. In the plots of intermediate yield, some of the rows showed considerable differences in rust damage; consequently, the harvested seed was a composite from plants suffering severe to moderate rust injury.

[1] Cooperative investigations between Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

[2] Pathologist, Division of Cereal Crops and Diseases.

[3] Dillman, A. C., and J. C. Brinsmade, Jr. Effect of spacing on the development of the flax plant. Jour. Amer. Soc. Agron. **30**: 267–278. 1930.

[4] Oil content computed on basis of 8 per cent moisture in the seed. Iodine number determined by refractive index. Analysis by E. P. Painter, Department of Agricultural Chemistry, North Dakota Agricultural Experiment Station.

The simple correlation coefficients between yield, weight of seed, oil content, iodine number, and stand are given in table 1.

High positive correlation coefficients were obtained between yield, weight of seed, and oil content, and high negative coefficients between each of these characters and iodine number of the oil. Correlation coefficients between stand and the other characters, namely, yield, weight of seed, oil content, and iodine number, were within the 1 per cent point and, with one exception, within the 5 per cent point, indicating a lack of significance.

A previous report[5] indicated that a light rust infestation, which reduced yields 25 per cent or less, had little effect on the oil content or quality. In the present tests, no rust-free Bison was available for comparative tests. However, the large seeds (6.2 grams per 1000) and high oil content (39.5 per cent) of the plot yielding at the rate of 16.8 bushels per acre were

TABLE 1.—*Correlation between yield as influenced by rust infection, and weight of 1,000 seeds, oil content, iodine number, and stand of Bison flax at Fargo, N. Dak., 1942*

Characters correlated	Correlation coefficient[a] with			
	Yield	Weight of 1,000 seeds	Oil content	Iodine number
Weight of 1,000 seeds	+0.97	.....	.....	.....
Oil content	+0.96	+0.94	.....	.....
Iodine number	−0.89	−0.83	−0.80	.....
Stand	+0.40	+0.40	+0.49	−0.31

[a] Coefficients above 0.56 are highly significant (beyond the 1 per cent point).

greater than usually obtained from Bison in non-rust years, indicating that moderate rust damage may not adversely affect seed size and oil content.

Dillman and Hopper[6] obtained samples of four varieties grown under many different environments where yield was largely determined by moisture and temperature. They found that, in general, those environmental conditions which reduced yield also reduced seed size, oil content, and iodine number.

In the present study, where differences in yield were due principally to rust infection, although the range in iodine number was small (178 to 183), significant negative correlations were obtained between iodine number and yield, seed size, and oil content. It would appear from these results that when yield is reduced by rust, the iodine number of the oil is not reduced as it is when the flax crop is adversely affected by drought or high temperatures.

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[5] Flor, H. H. Flax rust. N. Dak. Agr. Exp. Stat. Bimo. Bull. 3: 7–9. 1941.

[6] Dillman, A. C., and Hopper, T. H. Effect of climate on the yield and oil content of flaxseed and on the iodine number of linseed oil. U. S. Dept. Agr. Tech. Bull. No. 844. 1943.

BACTERIAL SOFT ROT OF SANSEVIERIA

J. G. Brown and Alice M. Boyle

(Accepted for publication October 10, 1943)

Sansevieria is an ornamental commonly cultivated in greenhouses and also planted outdoors in the warmest parts of the United States. Certain species of the genus, *S. guineensis* and *S. trifasciata* in particular, are grown for their fiber, bowstring hemp, in parts of the tropics. Use of the fiber of *Sansevieria* in a mixture of pineapple and other threads in the manufacture of pina and jusi cloths in the Philippine Islands is remembered by the senior writer. Ornamental planting of *Sansevieria* and commercial utilization of its fiber indicate considerable cultivation of the herb. Nevertheless, we find few references to its diseases and no mention of bacterial maladies affecting any plants of the more than forty species of the genus.

Plants of *Sansevieria trifasciata* growing in the horticultural greenhouse of the University of Arizona were all killed by disease in the winter of 1941–42, although they had been cared for in the usual manner. The leaves became yellowish-green to pale-yellow, developed soft, water-soaked spots at and above the soil line, toppled over sidewise (Fig. 1, *A*), and rotted off at the base (Fig. 1, *C, b, c*). The rootstocks became water-soaked, soft and straw-colored (Fig. 1, *C, d, e*), and the roots died and dried.

From the lesions on leaf and rootstock of several affected plants, colonies of a grayish-white bacterium were repeatedly obtained in pure culture. This bacterium, inoculated into healthy plants of *Sansevieria trifasciata* by the needle-stab method, and also sprayed on the same species, caused symptoms (Fig. 1, *B*) identical with the natural infection. The plants showed evidence of a "take" in 16 days and advanced symptoms of the disease, including many dead plants, in 43 days.

The cause of the soft rot of *Sansevieria* is a grayish-white, actively motile (by 1 and 2 polar flagella; 12-hr. culture, Casares-Gil), Gram-negative, nonspore-forming, slowly gelatine-liquifying, nitrate-reducing, milk-curdling (but nonpeptonizing) short rod, occurring singly or in chains of 2 to 6. Colonies on the surface of potato-dextrose agar plates are circular, elevated, smooth, moist-glistening, with well-defined margin. Growth in nutrient broth is abundant with formation of a pellicle and abundant flaky sediment. Acid curd appears in litmus milk, acid and gas in dextrose broth, and acid in l-arabinose, l-xylose, and raffinose; the reaction is alkaline in cellobiose, maltose, d-sorbitol, levulose, salicin, saccharose, glycerine, lactose, and mannitol. The carbon-source media, respectively, were made by adding 2 per cent by weight of the designated compound, before sterilization, to nutrient broth containing 3 g. of beef extract and 5 g. of peptone per liter.

The bacterium causing rot of *Sansevieria trifasciata, Erwinia carotovora, E. aroideae,* and *E. phytophthora,* which are all soft-rot organisms, are alike in being Gram-negative, aerobes, gelatine liquifiers, nitrate reducers, milk and litmus milk curdlers with acid reactions and no peptonization in the latter, white or grayish white color; acid producers in dextrose, l-arabinose, l-xylose, and raffinose; *E. carotovora* and *E. phytophthora* agree in

failing to produce NH_3 (reaction of *E. aroideae* in this medium appears to be unreported). The *Sansevieria* bacterium is further like *E. carotovora* in producing gas in dextrose, and both grow well on raw carrot; it agrees with *E. aroideae* in producing no indol; no gas in lactose, sucrose, l-xylose, raffinose, maltose, and levulose. Further similarity to *E. phytophthora* is the production of gas in dextrose.

The bacterium of *Sansevieria* soft rot is unlike the other pathogens mentioned above in motility, failure to reduce litmus, acid reaction in lactose and sucrose, and no acid in maltose, levulose, and mannitol; it is unlike both *Erwinia carotovora* and *E. phytophthora* and like *E. aroideae* in its lack of gas formation in lactose, sucrose, l-arabinose, l-xylose, raffinose, and levulose; it is unlike *carotovora* in causing no odor, no gas in lactose, sucrose, l-arabinose, l-xylose, raffinose, levulose, salicin, in growing poorly on steamed sweet potato; unlike *E. aroideae* in producing gas in lactose; further unlike *E. phytophthora* in producing no fringed colonies, and it does not brown or blacken raw potato.

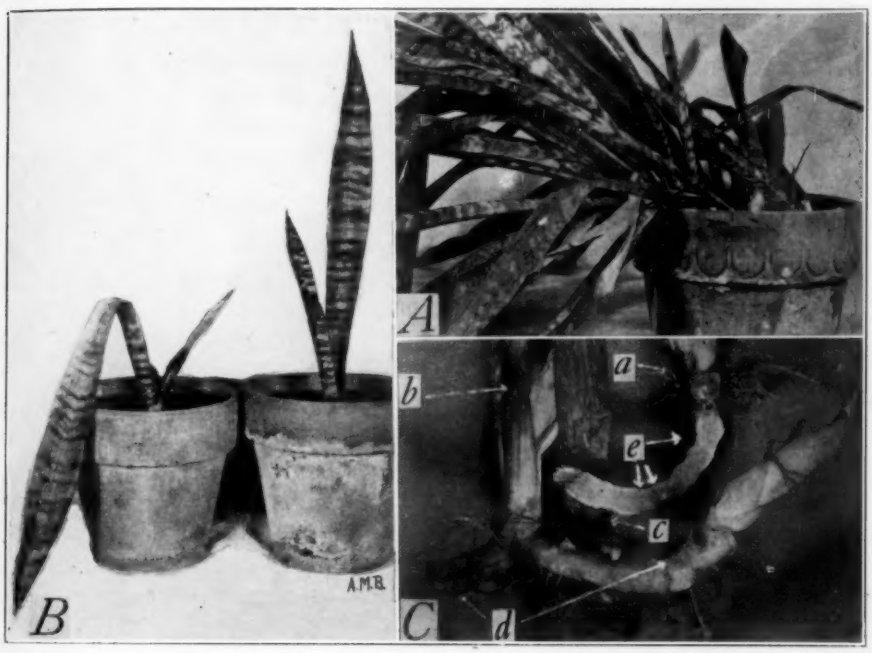

FIG. 1. *A.* Greenhouse plants of *Sansevieria trifasciata* Prain affected by bacterial soft rot. *B.* Inoculated plant, with drooping larger leaf and discolored drying smaller leaf (left). Control injected with sterile distilled water (right). *C.* Parts of naturally infected plants. *a.* Exterior of infected, water-soaked leaf base. *b.* Water-soaked, shrunken part of leaf with base of dead, dried and browned leaf at right. *c.* Lower part of leaf entirely rotted from rootstock. *d.* Exterior of water-soaked, discolored rootstocks to which dead roots and stubs of decayed roots are attached. *e.* Interior, soft-rotted tissues of rootstock.

The bacterium causing the rot of *Sansevieria trifasciata* herein described appears to belong to the group of intermediates mentioned by Stanley,[1] who reports that 19 cultures or 44.2 per cent of 43 cultures isolated from soft rots similarly are intermediate between *Erwinia carotovora* and *E. aroideae* assemblages.

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[1] Stanley, A. R. Physiologic and serologic studies of the soft-rot and colon group of bacteria. W. Va. Agr. Exp. Stat. Bull. 287. 1938.

PHYTOPATHOLOGICAL NOTES

The Pathogenicity of a Nonsporulating Basidiomycete on Grasses in Minnesota[1]—*Fusarium* sp., *Alternaria* sp., and a nonsporulating basidiomycete were isolated from several plants of crested wheatgrass (*Agropyron cristatum*), which had been severely injured by root rot at Clear Lake, Minnesota.[2] Earlier studies[3] had shown *Helminthosporium sativum* P. K. and B. and *Pythium graminicolum* Subr. to be important in causing root rot of this grass in Minnesota. But a nonsporulating basidiomycete had hitherto not been isolated. Pathogenicity of the basidiomycete was tested in the greenhouse by planting seeds of *Agropyron cristatum* (L.) Beauv., *Bromus inermis* Leyss., and *Festuca elatior* L. in steamed soil inoculated with cultures of the fungus grown on a corn meal-soil medium and in noninoculated steamed soil. The tests were made in greenhouses maintained

FIG. 1. *Agropyron cristatum* grown in the greenhouse at 85, 75, and 65 degrees F. in steamed soil inoculated with a nonsporulating basidiomycete and in noninoculated soil. The grass growing in inoculated soil is seen on the right at each temperature.

at about 65, 75, and 85 degrees F., respectively. At all these temperatures many seedlings of *Agropyron cristatum* failed to emerge from the inoculated soil and many seedlings were killed after emergence. The most severe injury occurred at 85° F., where only 16 per cent of the plants survived after 22 days (Fig. 1). *Bromus inermis* and *Festuca elatior* were not appreciably affected by the fungus (Table 1). The emergence and survival of these grasses on noninoculated, steamed soil were given a rating of 100 per cent for the purpose of comparison.

The fungus produces a dense white mycelial mat on potato-dextrose agar; the hyphae are from 3 to 6 μ in diameter and bear prominent clamp connections. Neither sclerotia nor spores have yet been observed, although the fungus was grown on several agar media, in water cultures, and in soil.

[1] Published as Paper 2107 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The material was submitted by Henry A. Johnson of the Soil Conservation Service.

[3] Andrews, Edward A. Seedling blight and root rot of grasses in Minnesota. Phytopath. 33: 234–239. 1943.

The organism grows faster and differs culturally from the low-temperature basidiomycete reported by Broadfoot and Cormack.[4] It also is readily distinguishable from cultures of *Typhula itoana* Imai,[5] and does not resemble other *Typhula* spp. described by Remsberg.[6]

The nonsporulating basidiomycete apparently is restricted to the crown of the plant. No lesions were seen on the roots of grasses attacked by this

TABLE 1.—*Susceptibility of Agropyron cristatum, Bromus inermis, and Festuca elatior to a nonsporulating basidiomycete at three temperatures in the greenhouse*

Grass	Emergence, and number of plants surviving 22 days after planting expressed as a percentage of the check[a]					
	65° F.		75° F.		85° F.	
	Emergence	Healthy plants	Emergence	Healthy plants	Emergence	Healthy plants
Agropyron cristatum	69	42	74	42	50	16
Bromus inermis	85	82	92	84	87	71
Festuca elatior	87	86	85	85	103	101

[a] One hundred and fifty seeds of each grass were planted at each temperature.

fungus nor was mycelium observed in any root. In reisolations pure cultures of the fungus were obtained consistently on potato-dextrose agar from the crown of infected plants but not from any other part of the root system.—EDWARD A. ANDREWS, University Farm, St. Paul, Minn.

The Production of Healthy Shoots by Wilted Tomato Plants.[1]—During the past few years the writer has had occasion to observe the behavior of hundreds of tomato plants, inoculated with *Fusarium bulbigenum* var. *lycopersici* and grown in the greenhouse under optimum conditions. Susceptible varieties, such as Bonny Best, usually wilted and died within 3 weeks after inoculation, but occasional plants, though wilting, produced new shoots with no symptoms of disease. These new shoots arose from meristematic regions near the base of the stem and continued to grow even after the main stem had died; they apparently were entirely normal and compared favorably with branches of healthy plants (Fig. 1). Such healthy branches from wilted plants have been observed for 7 weeks after their initial appearance; they produced flowers and even set fruit. The pathogen could invariably be reisolated from the roots and from the wilted main stem of the infected plant, both above and below the origin of the young branch. Only rarely was it isolated from the new shoot, and then only from tissues immediately adjacent to the old stem. Histologic studies showed that the fungus

[4] Broadfoot, W. C., and M. W. Cormack. A low-temperature basidiomycete causing early spring killing of grasses and legumes in Alberta. Phytopath. **31**: 1058. 1941.

[5] Cultures of *Typhula itoana* and the low-temperature basidiomycete were obtained from Ian W. Tervet, University of Minnesota.

[6] Remsburg, Ruth E. Studies in the genus *Typhula*. Mycologia **32**: 52–96. 1940.

[1] Published as Paper 2108 of the Journal Series of the Minnesota Agricultural Experiment Station.

was present in the vessels of all parts of the main stem, but was exceedingly difficult to find in the new shoot. Occasionally, however, a strand of mycelium could be seen in sections from the base of the branch.

The behavior of these plants is puzzling, for the young branch arises from meristematic tissues of the susceptible parent and its vessels are continuous with those of the crown and roots of the infected plant. The assumption is that the fungus would be free to grow into the vessels of the new shoot or at any rate the toxin produced by the fungus in the lower portions of the old

FIG. 1. An inoculated Bonny Best tomato plant showing wilted main stem and vigorous new shoot.

crown could be carried into the new shoot and cause it to wilt. The new shoot, however, appears resistant to the pathogen.—DAVID GOTTLIEB, Agricultural Experiment Station, University Farm, St. Paul, Minnesota.

Low-lime Bordeaux Mixture Controls Leaf Gall on Azaleas.—Azalea leaf gall (*Exobasidium vaccinii* (Fckl.) Wor.) is widespread in this country and Europe, attacking azaleas, rhododendrons, and species of *Vaccinium*. The galls appear on these hosts in the wild, as well as in the nursery and the garden. They have not assumed sufficient importance on cranberries or blueberries to warrant control experiments. On azalea, the galls are commonly so scattered as to excite curiosity only, but in wet seasons or in shaded gardens they may be so numerous as to cause some alarm. Previously pub-

lished accounts of the disease recommend for control the picking and burning of the galls and spraying with 8–8–100 Bordeaux mixture during the period of vegetative growth. The above strength of Bordeaux mixture has been recommended also for control of azalea leaf scorch (*Septoria azaleae* Vogl.), characterized by leaf spots and premature leaf drop.

In one of the writer's pecan experimental blocks near Albany, Georgia, 9 azaleas (*Rhododendron obtusum* (Planch.) Wils.) are planted around 2 of the trees. These pecan trees are the Schley variety, one that is very susceptible to pecan scab, so that 4 spray applications of Bordeaux mixture were necessary to control the disease. During the past 9 years these trees have been sprayed annually with low-lime-Bordeaux mixture as follows:

April 10 to 23—4–1–100 Bordeaux mixture
May 5 to 15—6–2–100 Bordeaux mixture
June 1 to 15—6–2–100 Bordeaux mixture
July 1 to 15—6–2–100 Bordeaux mixture

There was a light infection of the Exobasidium galls on the azalea plants when the spraying was first begun, but the galls soon disappeared and the plants have remained apparently free of all fungus diseases.

Adjacent to the 9 plants mentioned are 290 other azaleas, *Rhododendron obtusum* (Planch.) Wils., *R. macranthum* (Sweet), and *R. mucronulatum* (Blume), all of which carried the Exobasidium disease. A former owner preferred to pick the galls by hand, a practice recommended by local nurserymen; but this was a monotonous job and never really controlled the disease. Recently, however, a new owner took possession of this place and on May 10, 1943, sprayed the 290 diseased plants with 6–2–100 Bordeaux mixture. Fourteen days later most of the galls had become dry and detached from the shrubs. By June 1 the foliage had regained its green color, and some new growth was evident.

During the past 9 years the 9 azaleas have received 36 applications of Bordeaux mixture. There has been no evidence of injury either to flowers or plants. The plants were usually in full bloom when the 4–1–100 Bordeaux mixture was applied. It is possible that the disease could have been controlled by the single application of 4–1–100 Bordeaux mixture in April.

Accumulation of lime with an adverse effect on the pH of certain light soils of the South, might result from high-lime-Bordeaux mixture, such as the 8–8–100 formula. It has been observed also that this type of Bordeaux mixture, at least under certain conditions, may burn the flowers of Indica azaleas, although the leaves are not injured. If Bordeaux mixture is to be applied to azaleas, the 6–2–100 formula seems advisable.

The low-lime-Bordeaux mixture must be carefully prepared, as the margin of safety from copper injury is lower than that afforded by high-lime formulae.—JOHN R. COLE, Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

The Nature of Resistance of Flax to Fusarium lini.[1]—The nature of resistance of Bison C.I. 389 and Punjab C.I. 20 flax to the wilt fungus, *Fusarium lini* Bolley, was investigated by plating on nutrient medium numerous segments of the host taken at intervals after sowing in infested soil in the greenhouse. Cultures of 2 physiologic races of *F. lini* were added to sterilized soil before the seeds were planted. The seedlings were collected daily or every other day, cut into segments ½ to 2 cm. long, depending on size of plant, and plated on nutrient agar.

Bison, grown in soil inoculated with *Fusarium lini* race 6, to which Bison is susceptible, yielded *F. lini* from the primary roots the day the plants emerged, but the fungus was not isolated from the apex of the seedlings until 7 days after emergence. Thereafter, *F. lini* was present in the apex and throughout the entire plant; within 20 days all the plants were wilted. When Bison was grown in soil inoculated with race 11, to which it is resistant, it is significant that *F. lini* was not isolated from the upper parts, although the fungus was universally present in the primary roots near the ground line. In Punjab C.I. 20, which is susceptible to races 6 and 11, the fungus was prevalent throughout the plants a day or two after they emerged.

Thus, the resistant Bison actually does not exclude the pathogen, but confines the less virulent race 11 to the host root and crown tissues and delays the development and spread of the more virulent race 6 for several days. In the susceptible Punjab there is no such limitation or delay, and the fungus develops and spreads rapidly throughout the seedling tissues.

Other data obtained in these experiments indicate that certain varieties of flax may be infected with *Fusarium lini* and not wilt, or wilt only partly; in fact, partial wilting is rather common in certain varieties of flax. Also, plants may be killed to the ground line by *F. lini* and again give rise to healthy shoots. *F. lini* was obtained from segments of wilted branches on plants in which part of the plant remained green and turgid; but the fungus was not obtained from the green parts. Plants of a number of varieties and hybrids that wilted after they had begun to set bolls consistently yielded *F. lini* from the basal portion of the stems to the apex. These results indicate that the fungus must actually be present in the tissue of the stem or branches to induce wilting.—MAX SCHUSTER, Div. of Plant Pathology and Botany, University Farm, St. Paul, Minn.

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